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L2 ANSWER 1 OF 13 HCA COPYRIGHT 2002 ACS
136:178542 Quencher as Leaving Group: Efficient **Detection** of DNA-Joining Reactions. Sando, Shinsuke; **Kool, Eric T.** (Department of Chemistry, Stanford University, Stanford, CA, 94305-5080, USA). Journal of the American Chemical Society, 124(10), 2096-2097 (English) 2002. CODEN: JACSAT. ISSN: 0002-7863. Publisher: American Chemical Society.
AB In this short communication the authors describe a new fluorescence reporting strategy in which dabsyl, a well-known quencher, activates a hydroxyl group in a probe to convert it to a leaving group. When a nucleophilic phosphorothioate probe binds adjacent to a dabsyl quenched probe, autoligation occurs, releasing the quencher, and lighting up the probes. This signal change can be used to **detect** single nucleotide differences in DNA without enzymes or reagents. The fluorescence reporting strategy was demonstrated using probes complementary to adjacent sites in the H-ras gene. Early expts. have allowed the authors to conclude that the use of a dabsylate leaving group on probes enable facile **detection** of DNA strand-joining reactions.

L2 ANSWER 2 OF 13 HCA COPYRIGHT 2002 ACS
135:46387 Fluorescent nucleoside analogs and combinatorial fluorophore arrays comprising same. **Kool, Eric T.** (Research Corporation Technologies, Inc., USA). PCT Int. Appl. WO 2001044220 A2 20010621, 78 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US33681 20001213. PRIORITY: US 1999-461636 19991214.
AB The present invention provides fluorescent nucleoside analogs which comprise a fluorescent cyclic compd. joined to a carbon of a sugar mol. such as pentose, hexose, ribose or deoxyribose or analogs thereof in either an .alpha. or .beta. configuration. The subject compds. are useful as probes in the study of the structure and dynamics of nucleic acids and their complexes with proteins. In addn., the subject compds. are useful in any technique which uses labeled **oligonucleotides** for **detection**. Non-fluorescent spacer mols. in which a cyclohexane, cyclohexene, decalin, or benzene is joined to a carbon of a sugar moiety such as pentose, hexose, ribose or deoxyribose are also provided. Also provided are the 5'-dimethoxytrityl-3'-O-phosphoramidite derivs., suitable for incorporation into **oligonucleotides** by automated synthesizers. Combinatorial fluorophore array (CFA) libraries comprising oligomers of the subjects nucleoside analogs attached to one or more solid supports are also provided as are methods of selecting fluorophores from the CFA libraries. The present invention also provides **oligonucleotide** analogs comprising one or more of the subject nucleoside analogs in place of the DNA or RNA base.

L2 ANSWER 3 OF 13 HCA COPYRIGHT 2002 ACS
134:261580 Cloning as a tool for organic chemists. Pickert, John D.; Miller, Benjamin L. (Department of Chemistry, University of Rochester, Rochester, NY, 14627, USA). Comprehensive Natural Products Chemistry, Volume 7,

Searched by: Mary Hale 308-4258 CM-1 1E01

643-674. Editor(s): **Kool, Eric T.** Elsevier Science B.V.: Amsterdam, Neth. (English) 1999. CODEN: 69AGYB.

AB A review with 170 refs. Topics include: selection of a target DNA sequence; selection of an expression system; obtaining **oligonucleotides** in quantity using the polymerase chain reaction; restriction digests; dephosphorylation; **ligation**; transformation of DNA into Escherichia coli; screening for insert; verification of insert identity; large scale prodn. of protein in E. coli culture; case studies in cloning.

L2 ANSWER 4 OF 13 HCA COPYRIGHT 2002 ACS

133:69764 Highly sensitive multimeric nucleic acid probes synthesized by rolling circle replication. **Kool, Eric T.** (University of Rochester, USA). U.S. US 6077668 A 20000620, 54 pp., Cont.-in-part of U. S. Ser. No. 805,631. (English). CODEN: USXXAM. APPLICATION: US 1997-910632 19970813. PRIORITY: US 1993-47860 19930415; US 1995-393439 19950223; US 1997-805631 19970226.

AB The present invention provides **detectably** labeled RNA and DNA **oligonucleotide** multimers useful as diagnostic probes in medical, biol. and chem. applications. A method for synthesizing DNA and RNA **oligonucleotides**, **oligonucleotide** multimers, and analogs, preferably those that are **detectably** labeled, is also provided. **Oligonucleotide** synthesis is performed by combining a circular single-stranded **oligonucleotide** template with an effective polymerase and at least two types of nucleotide triphosphate, without the addn. of auxiliary proteins, to yield an **oligonucleotide** multimer comprising multiple copies of a repeated **oligonucleotide** sequence.

L2 ANSWER 5 OF 13 HCA COPYRIGHT 2002 ACS

133:325 Circular antisense **oligonucleotides** inhibit growth of chronic myeloid leukemia cells. Rowley, Peter T.; Kosciolek, Barbara A.; **Kool, Eric T.** (Department of Medicine and Division of Genetics, University of Rochester, Rochester, NY, USA). Molecular Medicine (New York), 5(10), 693-700 (English) 1999. CODEN: MOMEF3. ISSN: 1076-1551. Publisher: Springer-Verlag New York Inc..

AB Antisense represents a conceptually powerful method for regulating gene expression. However, antisense **oligonucleotides** developed to date manifest two serious limitations-nuclease susceptibility and non-specific hybridization. Circular **oligonucleotides** may be superior to conventional linear **oligonucleotides** in both respects. First, circular agents, having no ends, are exonuclease-resistant. Second, they bind to complementary strands of RNA and DNA with a higher affinity than corresponding linear agents. We assessed the activity of circular phosphodiester deoxynucleotides using chronic myeloid cell lines by targeting polypurine sequences. To represent cells having a bcr3/abl2-type junction, we used K562 cells. A circle targeting a bcr polypurine sequence 385 nucleotides 5' to the junction decreased the cell no. by day 5 with an IC50 of 9 .mu.M. To represent cells having a bcr2/abl2-type junction, we used BV173 cells. A circle targeting the bcr-abl junction itself decreased the cell no. by day 7 with an IC50 of 8 .mu.M. Control **oligonucleotides**, whether the same sequence uncircularized or circles with the same nucleotide compn. but in scrambled sequence, had little effect. Unlike linear agents, circles were stable when incubated in 10% serum. The amt. of bcr-abl protein **detected** by Western blotting using a specific anti-bcr-abl antibody at 24 h in antisense-treated BV173 cells was only 10% of that of cells treated with control circles, which demonstrates an antisense mechanism of action. Circular oligodeoxyribonucleotides (1) inhibit the accumulation of CML cells, (2) decrease the amt. of bcr-abl protein per cell, (3) have sequence-selective activity, and (4) are more active than linear **oligonucleotides** contg. only the base-pairing

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region.

L2 ANSWER 6 OF 13 HCA COPYRIGHT 2002 ACS
130:292382 High sequence fidelity in a non-enzymic DNA autoligation reaction. Xu, Yanzheng; **Kool, Eric T.** (Department of Chemistry, University of Rochester, Rochester, NY, 14627, USA). Nucleic Acids Research, 27(3), 875-881 (English) 1999. CODEN: NARHAD. ISSN: 0305-1048. Publisher: Oxford University Press.

AB The success of **oligonucleotide ligation** assays in probing specific sequences of DNA arises in large part from high enzymic selectivity against base mismatches at the **ligation** junction. We describe here a study of the effect of mismatches on a new non-enzymic, reagent-free method for **ligation** of **oligonucleotides.** In this approach, two **oligonucleotides** bound at adjacent sites on a complementary strand undergo autoligation by displacement of a 5'-end iodide with a 3'-phosphorothioate group. The data show that this **ligation** proceeds somewhat more slowly than **ligation** by T4 ligase, but with substantial discrimination against single base mismatches both at either side of the junction and a few nucleotides away within one of the **oligonucleotide** binding sites. Selectivities of >100-fold against a single mismatch are obsd. in the latter case. Expts. at varied concns. and temps. are carried out both with the autoligation of two adjacent linear **oligonucleotides** and with intramol. autoligation to yield circular "padlock" DNAs. Application of optimized conditions to discrimination of an H-ras codon 12 point mutation is demonstrated with a single-stranded short DNA target.

L2 ANSWER 7 OF 13 HCA COPYRIGHT 2002 ACS
130:219100 Highly sensitive multimeric nucleic acid probes prepared by rolling-circle synthesis. **Kool, Eric T.** (University of Rochester, USA). PCT Int. Appl. WO 9909216 A2 19990225, 103 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US16776 19980813. PRIORITY: US 1997-910632 19970813.

AB The present invention provides **detectably** labeled RNA and DNA **oligonucleotide** multimers useful as diagnostic probes in medical, biol. and chem. applications. A method for synthesizing DNA and RNA **oligonucleotides, oligonucleotide** multimers, and analogs, preferably those that are **detectably** labeled, is also provided. **Oligonucleotide** synthesis is performed by combining a circular single-stranded **oligonucleotide** template with an effective polymerase and at least 2 types of nucleotide triphosphate, without the addn. of auxiliary proteins, to yield an **oligonucleotide** multimer comprising multiple copies of a repeated **oligonucleotide** sequence. Particular advantages of the method of the invention over current methods for synthesizing repeating unit **oligonucleotide** multimers include (a) increased length of the multimeric product, (b) the ability to produce single-stranded multimers instead of only duplexes, and (c) the optional use of a primer to initiate synthesis, which can be constructed to carry a desired nonrepeating sequence.

L2 ANSWER 8 OF 13 HCA COPYRIGHT 2002 ACS
126:60292 Preparation of single-stranded circular **oligonucleotides.** **Kool, Eric T.** (Research Corporation Technologies, Inc., USA). PCT Int. Appl. WO 9630384 A1 19961003, 196 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,

SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US3757 19960321. PRIORITY: US 1995-413813 19950330.

AB The present invention provides single-stranded circular **oligonucleotides** each with at least one parallel binding (P) domain and/or at least one corresponding anti-parallel binding (AP) domain sepd. from each other by loop domains. When more than one P or AP domain is included in a circular **oligonucleotide** of the present invention, the addnl. P or AP domains can constitute loop domains for a pair of corresponding P and AP domains, and vice versa. The present invention further provides single-stranded circular **oligonucleotides** with at least one Hoogsteen anti-parallel (HAP) domain. Each P, AP and HAP domain has sufficient complementarity to bind one strand of a defined nucleic acid target wherein the P domain binds in a parallel manner to the target and the HAP or AP domain binds in an anti-parallel manner to the target. Moreover, the present single-stranded circular **oligonucleotides** can bind to both single-stranded and double-stranded target nucleic acids. The present invention also provides methods of making and using these **oligonucleotides** as well as kits and pharmaceutical compns. contg. these **oligonucleotides**. Single-stranded circular **oligonucleotides** is capable of binding to a target DNA or RNA and thereby regulating DNA replication, RNA transcription, protein translation, etc. They can be labeled for use such as probes to **detect** or isolate a target nucleic acid. They are resistant to exonucleases and thus superior to linear **oligonucleotides** for diagnostic and therapeutic applications. Thus, a circular **oligonucleotide** (I) antisense to the b2a2 chimeric bcr/abl junctional sequences of chronic myeloid leukemia genes, was prepd. by nonenzymic template directed cyclization of the corresponding linear precursor. I at 4 .mu.M in vitro was effective in inhibiting the proliferation of chronic myeloid leukemia K562 cells.

L2 ANSWER 9 OF 13 HCA COPYRIGHT 2002 ACS

125:51565 Circular **oligonucleotides**: new concepts in **oligonucleotide** design. **Kool, Eric T.** (Dep. Chem., Univ. Rochester, Rochester, NY, 14627, USA). Annu. Rev. Biophys. Biomol. Struct., 25, 1-28 (English) 1996. CODEN: ABBSE4. ISSN: 1056-8700.

AB A review, with 87 refs. Recent progress in the synthesis and properties of circular **oligonucleotides** as ligands for DNA and RNA and as templates for polymerase enzymes is described. Small synthetic circular DNAs, RNAs, and chimeric analogs ranging from 28 to 74 nucleotides in size have been synthesized with the use of nonenzymic **ligation** strategy. Some of these were designed to undergo triplex formation with single-stranded DNA and RNA targets, and many bind with affinities and sequence selectivities considerably greater than those seen for linear **oligonucleotides**. Design strategies and modes of binding are discussed in the light of possible use of such mols. as hybridization probes, mol. diagnostics, and sequence-specific inhibition of gene expression. Small circular **oligonucleotides** have also been shown to act as unusually efficient templates for DNA and RNA polymerases, which produce long repeating copies of the circular sequence by a rolling circle process.

L2 ANSWER 10 OF 13 HCA COPYRIGHT 2002 ACS

125:27656 Stem-loop **oligonucleotides** containing parallel and antiparallel binding domains, nucleic acid target determination or regulation, and pharmaceutical applications. **Kool, Eric T.** (Research Corporation Technologies, Inc., USA). U.S. US 5514546 A 19960507, 34 pp. (English). CODEN: USXXAM. APPLICATION: US 1993-115497 19930901.

AB The present invention provides stem-loop **oligonucleotides** contg. a double-stranded stem domain of at least about 2 base pairs and a single-stranded loop domain. The loop domains of the present

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oligonucleotides include at least one parallel binding (P) domain sepd. by at least about 3 nucleotides from a corresponding anti-parallel binding (AP) domain. Each P and corresponding AP domain of the present **oligonucleotides** can bind **detectably** to one strand of a defined nucleic acid target wherein the P domain binds in a parallel manner to the target and the corresponding AP domain binds in an anti-parallel manner to the target. The present stem-loop **oligonucleotides** can bind to both single-stranded and double-stranded target nucleic acids. The present invention also provides methods of using these **oligonucleotides** as well as kits and pharmaceutical compns. contg. these **oligonucleotides**.

L2 ANSWER 11 OF 13 HCA COPYRIGHT 2002 ACS

124:138841 Convergent DNA synthesis: a non-enzymic dimerization approach to circular oligodeoxynucleotides. Rubin, Ethel; Rumney, Squire, IV; Wang, Shaohui; **Kool, Eric T.** (Dep. Chemistry, Univ. Rochester, Rochester, NY, 14627, USA). Nucleic Acids Res., 23(17), 3547-53 (English) 1995. CODEN: NARHAD. ISSN: 0305-1048.

AB The authors report a novel convergent approach to the construction of circular DNA **oligonucleotides** from two smaller linear precursors. Circular DNAs 34-74 nucleotides (nt) in size are constructed non-enzymically in a single step from two half-length oligomers. A DNA template is used to assemble the constituent parts into a triple helical complex which brings the four reactive ends together for chem. **ligation** with BrCN/imidazole/Ni2+. A homodimerization reaction strategy is successfully used on a small scale to construct circles 42, 58 and 74 nt in size. In addn., a heterodimerization strategy is successfully used in two cases to construct circular 34mers from different 16mer and 18mer precursors. Measurement of preparative yields for one biol. active 34mer circle shows that the dimerization strategy gives a yield higher than that from conventional cyclization and nearly as high as that for a normally synthesized linear DNA, establishing that there is not necessarily a yield penalty for circle construction. Six addnl. preparative circle constructions, giving conversions of -33-85% from precursors to circular product, are also described. Convergent strategies allow the construction of medium and large size DNA mols. in higher yields than can be achieved by std. linear synthesis alone.

L2 ANSWER 12 OF 13 HCA COPYRIGHT 2002 ACS

121:223142 Circular RNA **oligonucleotides**. Synthesis, nucleic acid binding properties, and a comparison with circular DNAs. Wang, Shaohui; **Kool, Eric T.** (Dep. Chem., Univ. Rochester, Rochester, NY, 14627, USA). Nucleic Acids Res., 22(12), 2326-33 (English) 1994. CODEN: NARHAD. ISSN: 0305-1048.

AB The authors report the synthesis and nucleic acid binding properties of two cyclic RNA **oligonucleotides** designed to bind single-stranded nucleic acids by pyr.cntdot.pur.cntdot.pyr-type triple helix formation. The circular RNAs are 34 nucleotides in size and were cyclized using a template-directed nonenzymic **ligation**. To ensure isomeric 3'-5' purity in the **ligation** reaction, one nucleotide at the **ligation** site is a 2'-deoxyribose. Circle 1 is complementary to the sequence 5'-A12, and circle 2 is complementary to 5'-AAGAAAGAAAAG. Results of thermal denaturation expts. and mixing studies show that both circles bind complementary single-stranded DNA or RNA substrates by triple helix formation, in which two domains in a pyrimidine-rich circle sandwich a central purine-rich substrate. The affinities of these circles with their purine complements are much higher than the affinities of either the linear precursors or simple Watson-Crick DNA complements. For example, circle 1 binds rA12 (pH 7.0, 10 mM MgCl2, 100 mM NaCl) with a Tm of 48.degree.C and a Kd (37.degree.C) of 4.1 .times. 10-9 M, while the linear precursor of the circle binds with a Tm of 34.degree.C and a Kd of 1.2 .times. 10-6 M. The complexes of circle 2 are pH-dependent, as expected

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for triple helical complexes involving C(+)G.cntdot.C triads, and mixing plots for both circles reveal one-to-one stoichiometry of binding either to RNA or DNA substrates. Comparison of circular RNAs with previously synthesized circular DNA **oligonucleotides** of the same sequence reveals similar behavior in the binding of DNA, but strikingly different behavior in the binding of RNA. The cyclic DNAs show high DNA-binding selectivity, giving relatively weaker duplex-type binding with complementary RNAs. The relative order of thermodn. stability for the four types of triplex studied here is found to be DDD .mchgt. RRR > RDR .mchgt. DRD. The results are discussed in the context of recent reports of strong triplex dependence on RNA vs. DNA backbones. Triplex-forming circular RNAs represent a novel and potentially useful strategy for high-affinity binding of RNA.

L2 ANSWER 13 OF 13 HCA COPYRIGHT 2002 ACS
119:86063 Single-stranded circular **oligonucleotides**. **Kool, Eric T.** (Research Corp. Technologies, Inc., USA). PCT Int. Appl. WO 9217484 A1 19921015, 109 pp. DESIGNATED STATES: W: AU, BR, CA, FI, HU, JP, KR, NO; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US2480 19920326. PRIORITY: US 1991-675843 19910327.

AB Single-stranded circular **oligonucleotides** are provided, each with a parallel (P) and an antiparallel (AP) binding domain sepd. from each other by loop domains. Each P and AP domain has sufficient complementarity to bind to 1 strand of a defined nucleic acid target, wherein the P domain binds in a parallel manner to the target and the AP domain binds in an antiparallel manner to the target. Moreover, the single-stranded circular **oligonucleotides** can bind to both single- and double-stranded target nucleic acids. Also provided are methods using the **oligonucleotides** and antimicrbial pharmaceutical compns. contg. the **oligonucleotides**.

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L3 141 FILE MEDLINE
L4 270 FILE HCA

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L5          152 FILE BIOSIS
L6          121 FILE EMBASE
L7          141 FILE WPIDS

TOTAL FOR ALL FILES
L8          825 (LIGAT? OR LIGATUR?) AND (OLIGONUCLEOTIDE OR DNA OR RNA OR ?NUCL
                EOTIDE?) AND POLYMORPHISM?

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L10           6 FILE HCA
L11           6 FILE BIOSIS
L12           0 FILE EMBASE
L13          23 FILE WPIDS

TOTAL FOR ALL FILES
L14          35 L8 AND (DETECT? OR RADIO)(2W) LABEL?

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L15           0 FILE MEDLINE
L16           3 FILE HCA
L17           3 FILE BIOSIS
L18           0 FILE EMBASE
L19           8 FILE WPIDS

TOTAL FOR ALL FILES
L20          14 L14 AND (MUTATION? OR DELET? OR INSERT? OR GENETIC REARRANGE?)

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L21           0 FILE MEDLINE
L22           1 FILE HCA
L23           2 FILE BIOSIS
L24           0 FILE EMBASE
L25           6 FILE WPIDS

TOTAL FOR ALL FILES
L26           9 FLUORESCEN? AND L20

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PROCESSING COMPLETED FOR L26
L27           9 DUP REM L26 (0 DUPLICATES REMOVED)

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L27  ANSWER 1 OF 9  WPIDS (C) 2002 THOMSON DERWENT
AN   2002-106604 [14]   WPIDS
AB   WO 200202805 A UPAB: 20020301
     NOVELTY - A new method (M1) for the simultaneous sequence-specific
     identification and separation of polynucleotide fragments in a
     polynucleotide population (P1) comprises using restriction
     endonucleases that recognize degenerate bases in their recognition or
     cleavage sequence to produce P1 from transcribed RNA. The
     fragments are then ligated and amplified.
          DETAILED DESCRIPTION - A new method (M1) for the simultaneous
     sequence-specific identification and separation of polynucleotide
     fragments in a polynucleotide population (P1) comprises using
     restriction endonucleases that recognize degenerate bases in their
     recognition or cleavage sequence to produce P1 from transcribed
     RNA. The fragments are then ligated and amplified.
          In detail, M1 comprises:
          (a) reverse transcribing an RNA population to provide P1;
          (b) digesting P1 with one or more restriction endonucleases (RE1)

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having a degenerate recognition or cleavage sequence comprising a degenerate base, where the degenerate base is represented by the formula of Nm, where N is the extent of degeneracy, and m is the number of degenerate bases, to produce restriction fragments having Nm different single-stranded overhangs for each restriction endonuclease;

(c) **ligating** the restriction fragments having the same overhangs to a series of adapters whose sequences are complementary to the overhangs; and

(d) amplifying the restriction fragments.

INDEPENDENT CLAIMS are included for the following:

(1) a method (M2) for detecting **polymorphism** comprising:

(a) reverse transcribing an **RNA** population to provide a **polynucleotide** population;

(b) digesting the **polynucleotide** population with one or more RE1;

(c) **ligating** the restriction fragments having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(d) amplifying the restriction fragments;

(e) sequencing the amplified restriction fragments; and

(f) comparing the sequence of the amplified restriction fragments with the sequence of the same **polynucleotide** from a different source;

(2) a method (M3) for screening for interactions between a preselected protein and polypeptide fragments, comprising culturing the recombinant host cells of (1) under conditions which enable expression of correctly **inserted** restriction fragments by the host cell, and assaying the interaction of the polypeptide fragments encoded by the restriction fragments with the preselected protein;

(3) a method (M4) for detecting a change in the pattern of **RNA** expression in a tissue or cell associated with an internal or external factor comprising:

(a) determining the pattern of **RNA** expression in a first tissue or cell sample not subject to the internal or external change by a method comprising:

(i) reverse transcribing an **RNA** population to provide the **polynucleotide** population;

(ii) digesting a double-stranded cDNA library prepared from the **RNA** isolated from the first sample with one or more RE1, where m is 1-5;

(iii) **ligating** the restriction fragments produced from (ii) having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(iv) amplifying the restriction fragments; and

(v) displaying the pattern of **RNA** expression in the first sample;

(b) determining and optionally quantifying the pattern of **RNA** expression in a second tissue or cell sample subject to the physiological or pathological change by performing steps (a)-(d) of M1 with the second sample;

(c) digesting the restriction fragments obtained in step (b) with one or more further restriction endonucleases producing restriction fragments with single-stranded overhangs different from those produced in step (b); and

(d) comparing the first and the second displays to determine the effect of the internal or external factor on the pattern of **RNA** expression in the tissue;

(4) a method (M5) for diagnosis of a disease based on detecting a change in the pattern of **DNA** fragments in a disease tissue or cell sample comprising:

(a) determining the pattern of **DNA** fragments in the sample by:

(i) digesting a **DNA** isolated from the tissue or cell with one or more restriction RE1;

(ii) ligating the restriction fragments produced from (i) having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(iii) amplifying the restriction fragments; and

(iv) displaying the pattern of DNA fragments in the disease tissue or cell sample;

(b) determining the pattern of DNA fragments in a normal tissue or cell corresponding to the disease tissue by performing steps (a)-(d) of M1 with the normal tissue; and

(c) comparing the disease and the normal tissue or cell profile of DNA fragments;

(5) a method (M6) for detecting a change in the pattern of RNA expression in a cell sample in response to an external factor comprising steps (a) to (d) of M4;

(6) a method (M7) for constructing an expressed sequence tag (EST) library comprising:

(a) reverse transcribing an mRNA population isolated from a eukaryotic source to provide a polynucleotide population;

(b) digesting a double-stranded cDNA library prepared from the mRNA with one or more RE1;

(c) ligating the restriction fragments produced from (b) having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(d) amplifying the restriction fragments; and

(e) ligating the amplified restriction fragments into a suitable cloning vector;

(7) an isolated nucleic acid comprising an oligonucleotide selected from one of the 64 nucleotide sequences defined in the specification; and

(8) a recombinant host cell transformed with a vector comprising cloned polymerase chain reaction (PCR) obtained from M1.

USE - The method is useful for the simultaneous sequence-specific identification, separation and quantitative measurement of polynucleotide fragments in a polynucleotide population. The method is applicable in DNA fingerprinting, differential display of mRNA, mutation and polymorphism identification, drug screening, molecular taxonomy, and diagnosis of diseases such as heart disease, lung disease, kidney disease, neurodegenerative disease, liver disease, a disease of the reproductive system, or cancer.

The isolated nucleic acid molecule comprising an oligonucleotide selected from one of the 64 nucleotide sequences defined in the specification can be used in any of the methods described above (claimed).

ADVANTAGE - The method is rapid, quantitative and it determines the gene expression level without the requirement of sequence information.

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L27 ANSWER 2 OF 9 WPIDS (C) 2002 THOMSON DERWENT

AN 2001-381715 [40] WPIDS

AB WO 200142512 A UPAB: 20010719

NOVELTY - Hybridization assay, involves contacting distinct sample **polynucleotide** target (T1) and a standard **polynucleotide** target (T2) with a detectable sample probe (P1) specific for T1, and an independently detectable standard probe (P2) specific for T2, under hybridization conditions, and detecting the hybridization pattern of the probes.

DETAILED DESCRIPTION - Hybridization assay, involves contacting separate samples, each comprising T1 and T2, with P1 specific for T1 and P2 specific for T2, under hybridization conditions to produce a hybridization pattern comprising P1 signal and P2 signal at each sample, where P2 signal provides normalization control for the sample, and detecting the hybridization pattern.

Searched by: Mary Hale 308-4258 CM-1 1E01

INDEPENDENT CLAIMS are also included for the following:

(1) immobilizing (I) a **polynucleotide** probe, by combining the probe with a **polynucleotide** target stably associated with a surface of a solid support, where one of the probe and target is double-stranded comprising complementary strands, and the other is single-stranded having complementarity with one of the complementary strands, under conditions where the probe and target hybridize and the probe is thus immobilized; or combining the probe with a **polynucleotide** target stably associated with a surface of a solid support, where the probe and target are double-stranded comprising complementary strands and have complementarity, under conditions where the probe and target hybridize and the probe is immobilized;

(2) immobilizing and detecting (ID) a **polynucleotide** probe, by contacting a **polynucleotide** target which is stably associated with a surface of a solid support, with a double-stranded **polynucleotide** probe, and detecting specific hybridization of the probe to the target;

(3) a microarray (M) comprising a number of elements, where each element comprises a distinct sample **polynucleotide** target and a standard **polynucleotide** target; and

(4) a kit (K) for use in measuring hybridization, comprising (I) or a sample of T1 and T2, and P2.

USE - The method is useful for obtaining quantitative information about the genetic profile of a labeled nucleic acid sample, normalizing and quantitating hybridization reactions, for obtaining quantitative information about the physiological source of the sample nucleic acid, for comparing nucleic acid samples from two or more physiological sources to identify and quantify differences between the patterns, and thus providing data on the differential expression of a particular gene in the physiological sources being compared, in differential gene expression assays for the analysis of a diseased and normal tissue, analysis of a different tissue or subtissue types, and large scale correlation studies on the sequences, **mutations**, variants or **polymorphisms** among samples.

ADVANTAGE - The method provides quantitative information about each element of the microarray. Hybridization of the probe sequences and the standard sequences is not competitive, thereby reducing noise in the results.

Dwg.0/7

L27 ANSWER 3 OF 9 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-657558 [76] WPIDS
CR 2001-425661 [44]; 2002-010801 [67]
AB DE 10016348 A UPAB: 20020105

NOVELTY - Parallel sequencing of at least two different nucleic acids (NA), present in a mixture, is new.

DETAILED DESCRIPTION - Parallel sequencing of at least two different nucleic acids (NA), present in a mixture. At least one pair of primers is immobilized irreversibly on a surface and treated with an NA mixture containing molecules that can hybridize to both primers. The immobilized primers are extended, in complementary fashion, to form a counter strand, resulting in formation of secondary NA (sNA). The surface is freed of NA that is not irreversibly bound and the sNA amplified to form tertiary nucleic acid (tNA). Counter-strands (gtNA) of tNA are prepared and extended by a single **nucleotide** (nt) in which the 2'- or 3'-hydroxy is protected, and which is **detectably labeled**. The incorporated nt is identified, the protecting group removed and the label either removed or altered. The single-nt extension procedure is repeated until the required sequence information has been obtained.

INDEPENDENT CLAIMS are also included for the following:

(1) similar method in which tNA is treated so that it is bound to the surface only through the 5'-end of one strand, then cut with a type IIS

restriction enzyme (RE) to generate 3' or 5' overhangs, determining one or more bases in these overhangs, **ligating** linkers to the free ends (these linkers include a recognition site for type IIS RE), treating again with RE that recognizes the site introduced in the linker and repeating the process as required;

(2) apparatus for performing the new process;

(3) method for localized amplification of NA, comprising the new process as far as amplification to produce tNA; and

(4) surface-bound library of NA produced by the method for localized amplification of NA.

USE - The method is useful for detecting genes and transcripts (e.g. for expression analysis), identifying **mutations** and **polymorphisms**, and detecting organisms and viruses.

ADVANTAGE - The method provides highly parallel sequencing, requires relatively small amounts of **DNA**, can sequence long segments and does not require complex apparatus.

Dwg.0/13

L27 ANSWER 4 OF 9 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-025173 [03] WPIDS
CR 2001-032066 [01]
AB WO 200071753 A UPAB: 20020513

NOVELTY - Determining (I) whether a subject has or is predisposed to developing an arterial restenosis, comprises detecting a restenosis associated allele (RAA) in a nucleic acid sample from the subject, where detection of the restenosis allele indicates that the subject has or is predisposed to the development of a restenosis.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a kit (II) for determining the existence of or a susceptibility to developing a restenosis in a subject, comprising a first primer **oligonucleotide** that hybridizes 5' or 3' to an allele selected from allele 1 of any of the following markers: interleukin-1 alpha (IL-1A) (+4845), interleukin-1 beta (IL-1B) (-511), IL-1B (+3954), interleukin-1 receptor antagonist (IL-1RN) (VNTR (variable number tandem repeat)) and IL-IRN (+2018) or an allele in linkage disequilibrium with it;

(2) selecting (III) an appropriate therapeutic for an individual that has or is predisposed to developing a restenosis, by detecting whether the subject contains a RAA and selecting a therapeutic that compensates for a restenosis causative functional **mutation** that is in linkage disequilibrium with the RAA;

(3) determining (IV) the effectiveness of treating a subject that has or is predisposed to developing restenosis with a particular dose of a restenosis therapeutic, comprising:

(a) detecting the level, amount or activity of an IL-1 protein, or an IL-1 mRNA or **DNA** in a sample obtained from a subject;

(b) administering the particular dose of the particular therapeutic to the subject, detecting the level, amount or activity of an IL-1 protein; or an IL-1 mRNA or **DNA** in a sample obtained from a subject; and

(c) comparing the relative level, amount or activity obtained in step (a) with the level, amount or activity obtained in step (b);

(4) treating (V) or preventing the development of a restenosis in a subject comprising the steps of detecting the presence of a RAA and administering to the subject a therapeutic that compensates for causative **mutation** that is in linkage disequilibrium with the RAA;

(5) screening (VI) for a restenosis therapeutic, comprising:

(a) combining an IL-1 polypeptide or its bioactive fragment, an IL-1 binding partner and a test compound under conditions where, but for the test compound, the IL-1 protein and IL-1 binding partner are able to interact; and

(b) detecting the extend to which, in the presence of the test

compound, an IL-1 protein/IL-1 binding partner complex is formed, where an increase in the amount of complex formed by an agonist in the presence of the compound relative to in the absence of the compound or a decrease in the amount of complex formed by an antagonist in the presence of the compound relative to in the absence of the compound indicates that the compound is a restenosis therapeutic; and

(6) identifying (VII) a restenosis therapeutic, comprising:

(a) contacting an appropriate amount of a candidate compound with a cell or cellular extract, which expresses an IL-1 gene; and

(b) determining the resulting protein bioactivity, where a decrease of an agonist or an antagonist bioactivity in the presence of the compound as compared to the bioactivity in the absence of the compound indicates that the candidate is a restenosis therapeutic.

ACTIVITY - Vasotropic; antiinflammatory; hypotensive; anticoagulant; antilipemic. No supporting data is given.

MECHANISM OF ACTION - Modulators of interleukin-1 alpha and beta and interleukin-1 receptor antagonist activity.

USE - The method is useful for determining whether a subject has or is predisposed to developing an arterial restenosis (claimed). The restenosis associated allelic pattern permits the diagnosis of occlusive cardiovascular disorder. The diagnosis allows the most suitable treatment methods for restenosis to be used e.g. selecting therapies for initial vascular stenosis most likely to avoid subsequent stenoses.

The detection methods identify restenosis therapeutics, agonists and antagonists, (proteins, peptides, peptidomimetics, small molecules or nucleic acids e.g. anti-sense, ribozyme and triplex nucleic acids) which are used to treat restenosis.

Dwg.0/10

L27 ANSWER 5 OF 9 WPIDS (C) 2002 THOMSON DERWENT
AN 2000-136988 [12] WPIDS
AB WO 9967414 A UPAB: 20000308

NOVELTY - A method for identifying a selected **nucleotide** in a nucleic acid uses a mobile solid support for either a primer, one component of a **ligation** reaction or a capture **oligonucleotide**.

DETAILED DESCRIPTION - The nucleic acid is hybridized with a primer that

(a) is linked at its 5' end to a detectably tagged mobile solid support; and

(b) includes a region complementary to a section of directly 3' of, and adjacent to, selected nt.

A primer extension reaction is performed to incorporate a **detectably labeled**, chain-terminating **nucleotide** and the presence or absence of the label in the hybridization product is determined. The presence and identity of the label indicates which chain-terminating **nucleotide** has been incorporated and the identity of the complementary to the selected **nucleotide**, and identifies the selected **nucleotide** in a nucleic acid.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of determining a selected **nucleotide polymorphism** in genomic **DNA** treated to reduce viscosity, comprising;

(a) amplifying the genomic **DNA** using a polymerase chain reaction (PCR);

(b) contacting the PCR product with a nucleic acid linked at its 5' end to a detectably tagged mobile solid support, the nucleic acid comprises a region complementary to a section of one strand of the PCR product which is directly 5' to the selected **nucleotide**, under hybridization conditions;

(c) performing a primer extention reaction with the hybridization product and a **detectably labeled**, identified

chain-terminating **nucleotide**;

(d) detecting the presence or absence of a label incorporated into the hybridization product, indicating the identity of the **nucleotide** complementary to the selected **nucleotide**; and

(e) comparing the identity of the selected **nucleotide** with a non-polymorphic **nucleotide**, a different identity indicates a **polymorphism** of that selected **nucleotide**;

(2) a method for determining a selected **nucleotide polymorphism** in genomic **DNA** treated to reduce viscosity, comprising the method of (1) where step (a) uses as a primer an **oligonucleotide** comprising a first region having a T7 **RNA** polymerase promoter and a second region complementary to a section of one strand of the nucleic acid which is directly 5' of the selected **nucleotide**, and **RNA** polymerase to amplify one strand into cRNA and reverse transcriptase to amplify a second strand complementary to the first, and amplifying to form an amplification product;

(3) a method of determining a selected **nucleotide polymorphism** in genomic **DNA** treated to reduce viscosity, comprising

(a) contacting the genomic **DNA** with a first primer linked at its 5' end to a detectably tagged mobile solid support, the primer comprises a region complementary to a section of one strand of the genomic **DNA** directly 5' of and adjacent to the selected **nucleotide** ; and

(b) performing steps (c), (d) and (e) of method (1);

(4) a method of identifying a selected **nucleotide** in a first nucleic acid comprising:

(a) contacting the first nucleic acid with a second nucleic acid, linked at its 5' end to a detectably tagged mobile solid support, which comprises a region complementary to a section directly 3' of the selected **nucleotide**, and terminates at its 3' end in a test **nucleotide** positioned to base-pair with the selected **nucleotide**, and a third, **fluorescently** labeled nucleic acid, which comprises a region complementary to a section directly 5' of and adjacent to the selected **nucleotide**;

(b) adding to the hybridization product a ligase; and

(c) detecting the presence or absence of the **fluorescent** label, after dissociation of the hybridized nucleic acids, in the nucleic acid linked to the solid support, the labels presence indicating the **ligation** to the third nucleic acid to the second nucleic acid linked to the mobile solid support, the identity of the test **nucleotide** in the second nucleic acid indicating the identity of the selected **nucleotide**;

(5) a method of quantitating expression of a selected nucleic acid in a sample, comprising:

(a) contacting message nucleic acid isolated from a selected source with a nucleic acid probe linked at its 5' end to a detectably tagged mobile support, where the nucleic probe comprises a region complementary to a section of the selected nucleic acid;

(b) performing the detection method of one of the methods above; and

(c) quantitating the **fluorescence** indicating the quantity of the selected nucleic acid in the sample; and

(6) a method of detecting a result from an identification reaction to identify a selected **nucleotide** in a target nucleic acid, comprising:

(a) contacting a target **oligonucleotide** comprising a first complementarity region and a second complementarity region, where the second region is 5' of the first, and the first is complementary to a section of the target nucleic acid directly 3' of and adjacent to the selected **nucleotide**, with a sample comprising the target nucleic acid;

(b) performing a selected identification reaction with the first hybridization product to determine the identity of the selected **nucleotide** where a selectively labeled detection product comprising the second region of the target **oligonucleotide** can be formed;

(c) isolating the detection product by contacting the detection product with a capture **oligonucleotide** covalently coupled to a mobile solid support; and

(d) detecting and/or identifying the label of the labeled detection product in the second hybridization product, the presence and/or identity of the label indicating the identity of the selected **nucleotide** in the target nucleic acid.

USE - The method is used to detect single nucleotide polymorphisms in genomic or amplified nucleic acid, e.g. for genotyping (detecting disease-associated mutations or pathogens), determining parentage, detecting or identifying pathogens and for determining differential gene expression.

ADVANTAGE - Exploiting the matrixing potential of mobile solid support (having multiple dye color/concentration capabilities) overcomes the limitations of known (immobilization-based) genetic bit analysis methods, i.e. low density on a two-dimensional support; photobleaching; auto-fluorescence of supports, inconsistent coupling of oligonucleotides to glass, and lack of flexibility, and expense, in producing new fixed arrays.

Dwg.0/0

L27 ANSWER 6 OF 9 WPIDS (C) 2002 THOMSON DERWENT
AN 1999-633727 [54] WPIDS
AB WO 9951621 A UPAB: 20020528

NOVELTY - Method for hybridizing two nucleic acids (NA) in which at least one NA comprises a minor groove binder (MGB)-**oligonucleotide** conjugate (A), is new. MGB is a molecule of 150-2000 D that binds in a non-intercalating manner to the minor groove of a double-stranded NA with association constant over 103 M-1.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(a) method of primer extension in which at least one primer is (A);

(b) differentiating between NAs that differ by a single **nucleotide** (nt) by reacting a target NA with at least two (A), one being perfectly complementary with the target and the other having a single mismatch, and determining hybridization strength of each (A);

(c) method similar to (b) but using single (A) of known sequence and at least two NAs, one the perfect complement of (A) and the other having a single nt mismatch;

(d) method for **ligating** two or more **oligonucleotides** (ON), each hybridized to adjacent positions on a target in which at least one ON is an (A);

(e) **oligonucleotide** probes (ONP) or use in mismatch discrimination and having one or more **detectable labels** that is an (A);

(f) (A) for use as a primer, having a 3'-end extendable by a polymerase;

(g) compositions and kits containing ONP of (e) or (A) of (f);

(h) primer-dependent nt sequencing using (A) as primer;

(i) method of examining gene expression using an array of ONP, at least one being an (A);

(j) method for identifying one or more **mutations** in a gene using an array of ONP as above;

(k) (A) that hybridize to a target NA to form a hybrid the melting point (m. p.) of which is independent of base composition;

(l) method of cDNA synthesis using (A) as primer; and

(m) method for detecting a target NA by hybridization to (A) that is

perfectly complementary to a target that differs by only a single nt from other non-target NAs.

ACTIVITY - None given.

MECHANISM OF ACTION - None given.

USE - Hybridization with (A), particularly where this is a probe or primer, is used in primer extension (amplification) reactions; to identify single-**nucleotide** (nt) mismatches; in ligase reactions; in sequencing; for analysis of gene expression and detection of **mutations**; for detecting target nucleic acids (especially for diagnosis or forensic analysis, e.g. to detect human immune deficiency virus or to differentiate between its subtypes, including those that are resistant to antiviral agents) and for cDNA synthesis.

ADVANTAGE - (A) form hybrids with complementary target sequences of very high stability, so even short probes, e.g. 8-mers, are highly specific and efficient. (A) also improve the discriminatory capacity of short **oligonucleotides**, providing better detection of single-base mismatches, and the speed (more rapid annealing to target) and versatility of assays are increased. Short primers are easier, and less expensive, to produce.

Dwg.0/5

L27 ANSWER 7 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2000:95825 Document No.: PREV200000095825. **DNA** technology in the clinical laboratory: Overview. Kiechle, Frederick L. (1). (1) Department of Clinical Pathology, William Beaumont Hospital, 3601 W 13 Mile Rd, Royal Oak, MI, 48073-6769 USA. Archives of Pathology & Laboratory Medicine, (Dec., 1999) Vol. 123, No. 12, pp. 1151-1153. ISSN: 0363-0153. Language: English. Summary Language: English.

AB Objectives.-To review the advances in clinically useful molecular biological techniques and to identify their applications in clinical practice, as presented at the Eighth Annual William Beaumont Hospital Symposium. Data Sources.-The 10 manuscripts submitted were reviewed, and their major findings were compared with literature on the same topic. Study Selection.-Two manuscripts addressed specimen (nucleic acid) stability, 2 described novel analytic approaches, 3 discussed detection of B- or T-cell clonality in lymphoproliferative disorders, and 3 reported the frequency of a variety of genetic **polymorphisms** found in cardiac disorders. Data Synthesis.-**DNA** from dried blood spots is stable and may be purified rapidly for amplification and **mutation** analysis. **RNA** is much less stable, and a variety of methods may be used to reduce ribonuclease degradation of enteroviral **RNA**. False-negative reactions may be reduced by genomic amplification of **ligated** padlock probes by cascade rolling circle or polymerase chain reaction. A multiplex polymerase chain method using **fluorescence**-labeled products that separate both the wild-type and mutant hemochromatosis gene alleles by capillary gel electrophoresis represents another approach for detecting the 2 major missense **mutations** (C282Y and H63D) in hemochromatosis. Southern blotting and polymerase chain reaction have been used to detect B- and T-cell clonality in lymphoproliferative diseases, including mantle cell lymphoma and lymphoma of the breast. Genetic **polymorphisms** in a variety of coagulation factors and platelet glycoprotein IIIa are associated with ischemic heart disease. Conclusions.-As the Human Genome Project continues to define disease-associated **mutations**, the number of clinically useful molecular pathologic techniques and assays will expand. Clinical outcome analysis is still required to document a decrease in the patient's length of stay to offset the cost of introducing molecular biological assays in the routine clinical pathology laboratory.

L27 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1998:271311 Document No.: PREV199800271311. A homogeneous, ligase-mediated **DNA** diagnostic test. Chen, Xiangning; Livak, Kenneth J.; Kwok,

Searched by: Mary Hale 308-4258 CM-1 1E01

Pui-Yan (1). (1) Div. Dematol., Washington Univ. Sch. Med., Saint Louis, MO 63110 USA. Genome Research, (May, 1998) Vol. 8, No. 5, pp. 549-556. ISSN: 1088-9051. Language: English.

AB Single-**nucleotide** variations are the most widely distributed genetic markers in the human genome. A subset of these variations, the substitution **mutations**, are responsible for most genetic disorders. As single **nucleotide polymorphism** (SNP) markers are being developed for molecular diagnosis of genetic disorders and large-scale population studies for genetic analysis of complex traits, a simple, sensitive, and specific test for single **nucleotide** changes is highly desirable. In this report we describe the development of a homogeneous **DNA** detection method that requires no further manipulations after the initial reaction is set up. This assay, named dye-labeled **oligonucleotide ligation** (DOL), combines the PCR and the **oligonucleotide ligation** reaction in a two-stage thermal cycling sequence with **fluorescence** resonance energy transfer (FRET) detection monitored in real time. Because FRET occurs only when the donor and acceptor dyes are in close proximity, one can infer the genotype or **mutational** status of a **DNA** sample by monitoring the specific **ligation** of dye-labeled **oligonucleotide** probes. We have successfully applied the DOL assay to genotype 10 SNPs or **mutations**. By designing the PCR primers and **ligation** probes in a consistent manner, multiple assays can be done under the same thermal cycling conditions. The standardized design and execution of the DOL assay means that it can be automated for high-throughput genotyping in large-scale population studies.

L27 ANSWER 9 OF 9 HCA COPYRIGHT 2002 ACS

127:215947 Detection of nucleic acid sequence differences using the ligase detection reaction with addressable array. Barany, Francis; Barany, George; Hammer, Robert P.; Kempe, Maria; Blok, Herman; Zirvi, Monib (Cornell Research Foundation, Inc., USA; University of Minnesota; Louisiana State University; Barany, Francis; Barany, George; Hammer, Robert P.; Kempe, Maria; Blok, Herman; Zirvi, Monib). PCT Int. Appl. WO 9731256 A2 19970828, 124 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US1535 19970205. PRIORITY: US 1996-11359 19960209.

AB The present invention describes a method for identifying one or more of a plurality of sequences differing by one or more single base changes, **insertions**, **deletions**, or translocations in a plurality of target **nucleotide** sequences. The method includes a **ligation** phase, a capture phase, and a detection phase. The **ligation** phase utilizes a **ligation** detection reaction between one **oligonucleotide** probe, which has a target sequence-specific portion and an addressable array-specific portion, and a second **oligonucleotide** probe, having a target sequence-specific portion and a **detectable label**. After the **ligation** phase, the capture phase is carried out by hybridizing the **ligated oligonucleotide** probes to a solid support with an array of immobilized capture **oligonucleotides** at least some of which are complementary to the addressable array-specific portion. Following completion of the capture phase, a detection phase is carried out to **detect** the **labels** of **ligated oligonucleotide** probes hybridized to the solid support. The **ligation** phase can be preceded by an amplification process. The present invention also relates to a kit for practicing this method, a method of forming arrays on solid supports, and the supports themselves.

Searched by: Mary Hale 308-4258 CM-1 1E01

L28 0 FILE MEDLINE
L29 6 FILE HCA
L30 6 FILE BIOSIS
L31 0 FILE EMBASE
L32 23 FILE WPIDS

TOTAL FOR ALL FILES
L33 35 L14 NOT L2

=> dup rem l33
PROCESSING COMPLETED FOR L33
L34 34 DUP REM L33 (1 DUPLICATE REMOVED)

=> d 1-34 cbib abs

L34 ANSWER 1 OF 34 HCA COPYRIGHT 2002 ACS
136:274240 Methods and apparatus for nucleic acid analysis. Drmanac, Radoje (USA). U.S. Pat. Appl. Publ. US 20020042048 A1 20020411, 37 pp., Cont.-in-part of U.S. 6,297,006. (English). CODEN: USXXCO. APPLICATION: US 1997-892503 19970714. PRIORITY: US 1997-784747 19970116; US 1997-812951 19970304.
AB The present invention provides a method for detecting a target nucleic acid species including the steps of providing an array of probes affixed to a substrate and a plurality of labeled probes. Each labeled probe is selected to have a first nucleic acid sequence which is complementary to a first portion of a target nucleic acid and wherein the nucleic acid sequence of at least one probe affixed to the substrate is complementary to a second portion of the nucleic acid sequence of the target. The invention relates to applying a target nucleic acid to the array under suitable conditions for hybridization of probe sequences to complementary sequences. The method further involves introduction of labeled probe to the array, hybridizing a probe affixed to the substrate to the target nucleic acid, hybridizing the labeled probe to the target nucleic acid, affixing the labeled probe to an adjacently hybridized probe in the array and **detecting** the **labeled** probe affixed to the probe in the array. The invention further relates to covalent joining of the immobilized probes to labeled probes that are immediately adjacent to the immobilized probe on the target sequence; removing any non-**ligated** labeled probes; detecting the presence of the target nucleic acid by detecting the presence of said labeled probe attached to the immobilized probes.

L34 ANSWER 2 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2002-217201 [27] WPIDS
AB WO 200212567 A UPAB: 20020429
NOVELTY - Diagnosing (M1) a genetic susceptibility for a disease (D), condition, or disorder in a subject comprising obtaining a biological sample containing nucleic acid from the subject, and analyzing the nucleic acid to detect the presence or absence of a single **nucleotide polymorphism** (SNP) in the vHL gene, where the SNP is associated with a genetic predisposition for a disease, condition or disorder, is new.

DETAILED DESCRIPTION - Diagnosing (M1) a genetic susceptibility for a disease (D), condition, or disorder in a subject comprising obtaining a biological sample containing nucleic acid from the subject, and analyzing the nucleic acid to detect the presence or absence of a single **nucleotide polymorphism** (SNP) in the vHL gene, where the SNP is associated with a genetic predisposition for a disease, condition or disorder, where (D) is selected from colon cancer, hypertension (HTN),

non-insulin dependent diabetes mellitus (NIDDM), atherosclerotic peripheral vascular disease due to HTN or NIDDM, cerebrovascular accident due to HTN or NIDDM, cataracts due to HTN, hypertensive cardiomyopathy, myocardial infarction due to HTN or NIDDM, end stage renal disease (due to HTN, NIDDM, focal segmental glomerular sclerosis or insulin dependent diabetes mellitus), ischemic cardiomyopathy, ischemic cardiomyopathy with NIDDM, atrial fibrillation without valvular disease, alcohol abuse, alcoholic cirrhosis, anxiety, asthma, chronic obstructive pulmonary disease, cholecystectomy, degenerative joint disease, end stage renal disease and frequent de-clots, and seizure disorder, is new.

INDEPENDENT CLAIMS are also included for the following:

(1) an isolated **polynucleotide** (I) comprising at least 10 contiguous **nucleotides** of a sequence (S) comprising 14543 base pairs fully defined in the specification, or its complement and containing at least one SNP at position 520 or 638 of (S), where the SNP is associated with (D);

(2) a kit (II) comprising (I) and instructions for using (I) for detecting the presence or absence of at least one SNP in the nucleic acid; and

(3) treatment (M2) or prophylaxis in a subject by obtaining a sample of biological material containing nucleic acid from a subject, analyzing the nucleic acid to detect the presence or absence of at least one SNP in (S) or its complement associated with (D), and treating the subject for (D).

ACTIVITY - Cytostatic; hypotensive; antidiabetic; vasotropic; antiasthmatic; antiarteriosclerotic; ophthalmological; cardiant; tranquilizer; hepatotropic; antialcoholic. No supporting data is given.

MECHANISM OF ACTION - None given.

USE - M1 is useful for diagnosing a genetic susceptibility for (D). M2 is useful for treatment or prophylaxis of (D) in a subject (claimed). Dwg.0/1

L34 ANSWER 3 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2002-241578 [29] WPIDS
AB WO 200208468 A UPAB: 20020508

NOVELTY - An isolated **polynucleotide** (I) comprising at least 10 contiguous **nucleotides** of a 2205 **nucleotide** sequence (S1), given in the specification, or its complement, and containing at least one single **nucleotide polymorphism** (SNP) at position 216 or 563 of S1 (transforming growth factor (TGF)- beta 1), where SNP is associated with a disease, condition or disorder, is new.

DETAILED DESCRIPTION - An isolated **polynucleotide** (I) comprising at least 10 contiguous **nucleotides** of a 2205 **nucleotide** sequence (S1), given in the specification, or its complement, and containing at least one single **nucleotide polymorphism** (SNP) at position 216 or 563 of S1 (transforming growth factor (TGF)- beta 1), where SNP is associated with a disease, condition or disorder, is new. SNP is associated with a disease, condition or disorder (D) including breast cancer, prostate cancer stage D, colon cancer, lung cancer, hypertension, atherosclerotic peripheral vascular disease due to hypertension, cerebrovascular accident due to hypertension, cataracts due to hypertension, hypertensive cardiomyopathy, myocardial infarction due to hypertension, end stage renal disease due to hypertension, non-insulin dependent diabetes mellitus, atherosclerotic peripheral vascular disease due to non-insulin dependent diabetes mellitus, cerebrovascular accident due to non-insulin dependent diabetes mellitus, ischemic cardiomyopathy, ischemic cardiomyopathy with non-insulin dependent diabetes mellitus, myocardial infarction due to non-insulin dependent diabetes mellitus, atrial fibrillation without valvular disease, alcohol abuse, anxiety, asthma, chronic obstructive pulmonary disease, cholecystectomy, degenerative joint disease, end stage renal disease and frequent de-clots, end stage renal disease due to focal

segmental glomerular sclerosis, end stage renal disease due to insulin dependent diabetes mellitus, and seizure disorder.

INDEPENDENT CLAIMS are also included for the following:

(1) diagnosing (M1) a genetic susceptibility for a disease, condition, or disorder in a subject, by obtaining a biological sample containing nucleic acid from the subject, and analyzing the nucleic acid to detect the presence or absence of SNP in TGF- beta 1 gene, where SNP is associated with a genetic predisposition for (D);

(2) a kit (K) comprising (I), and instructions for detecting the presence or absence of at least one SNP in the nucleic acid; and

(3) treatment or prophylaxis (M2) in a subject, which involves obtaining a sample of biological material containing nucleic acid from a subject, analyzing the nucleic acid to detect the presence or absence of at least one SNP in S1 or its complement, associated with (D), and treating the subject for (D).

ACTIVITY - Cytostatic; Hypotensive; Cardiant; Vasotropic; Antiarteriosclerotic; Antidiabetic; Nephrotropic; Antialcoholism; Tranquilizer; Antiasthmatic.

MECHANISM OF ACTION - Gene therapy.

No biological data is given.

USE - M1 is useful for diagnosing genetic susceptibility for (D). (I) is useful for designing prophylactic treatment regimes for patients determined to have an increased susceptibility to (D).

Dwg.0/0

L34 ANSWER 4 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2002-188635 [24] WPIDS
CR 2001-451916 [48]
AB WO 200208467 A UPAB: 20020416

NOVELTY - Diagnosing a genetic susceptibility for a disease, condition, or disorder in a subject comprises analyzing a nucleic acid in a biological sample to detect the presence of a single **nucleotide polymorphism** in the endothelial consecutive nitric oxide synthase (ecNOS) gene.

DETAILED DESCRIPTION - Diagnosing a genetic susceptibility for a disease, condition, or disorder in a subject comprises analyzing a nucleic acid in a biological sample to detect the presence of a single **nucleotide polymorphism** in the endothelial consecutive nitric oxide synthase (ecNOS) gene. The single **nucleotide polymorphism** is associated with a disease, condition or disorder selected from breast cancer, lung cancer, prostate cancer, non-insulin dependent diabetes, end stage renal disease due to non-insulin dependent diabetes, hypertension, end stage renal disease due to hypertension, myocardial infarction, colon cancer, hypertension, atherosclerotic peripheral vascular disease due to hypertension, cerebrovascular accident due to hypertension, cataracts due to hypertension, cardiomyopathy with hypertension, myocardial infarction due to hypertension, non-insulin dependent diabetes mellitus, atherosclerotic peripheral vascular disease due to non-insulin dependent diabetes mellitus, cerebrovascular accident due to non-insulin dependent diabetes mellitus, ischemic cardiomyopathy, ischemic cardiomyopathy with non-insulin dependent diabetes mellitus, myocardial infarction due to non-insulin dependent diabetes mellitus, atrial fibrillation without valvular disease, alcohol abuse, anxiety, asthma, chronic obstructive pulmonary disease, cholecystectomy, degenerative joint disease, end stage renal disease and frequent de-clots, end stage renal disease due to focal segmental glomerular sclerosis, end stage renal disease due to insulin dependent diabetes mellitus, and seizure disorder.

INDEPENDENT CLAIMS are also included for the following:

(1) an isolated **polynucleotide** comprising at least 10 contiguous **nucleotides** of a fully defined sequence of 3585 base pairs (bp) (S1), or its complement, and containing at least one single

nucleotide polymorphism at position 2548, 2684, 2701, or 2843 of S1;

(2) a kit comprising at least one isolated **polynucleotide** of at least 10 contiguous **nucleotides** of (I) or its the complement, and containing at least one single **nucleotide polymorphism** associated with a disease, condition, or disorder stated above;

(3) a kit comprising at least one **polynucleotide** of at least 10 contiguous **nucleotides** of S1 or its complement, where the 3' end of the **polynucleotide** is immediately 5' to a single **nucleotide polymorphism** site associated with a genetic predisposition to disease, condition, or disorder stated above; and

(4) a method for the treatment or prevention in a subject comprising obtaining a sample of biological material containing nucleic acid from a subject, analyzing the nucleic acid to detect the presence of at least one single **nucleotide polymorphism** in (I) or its complement associated with a disease, condition, or disorder stated above.

ACTIVITY - Cytostatic; Anti-diabetic; nephrotropic, hypotensive; cardiant; Anti-atherosclerotic, Vasoconstrictor; Ophthalmalogical; Tranquilizer; Anxiolytic; Anti-asthmatic; Sedative; Antiinflammatory; Anticoagulant; Osteopathic.

MECHANISM OF ACTION - No suitable data is given in the specification.

USE - The method is useful for diagnosing a genetic predisposition to a disease associated with single **nucleotide polymorphisms**, for designing a treatment regimen for a patient having a disease, condition or disorder caused either directly or indirectly by the presence of one or more single **nucleotide polymorphisms**.

Dwg.0/0

L34 ANSWER 5 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2002-106604 [14] WPIDS
AB WO 200202805 A UPAB: 20020301

NOVELTY - A new method (M1) for the simultaneous sequence-specific identification and separation of **polynucleotide** fragments in a **polynucleotide** population (P1) comprises using restriction endonucleases that recognize degenerate bases in their recognition or cleavage sequence to produce P1 from transcribed **RNA**. The fragments are then **ligated** and amplified.

DETAILED DESCRIPTION - A new method (M1) for the simultaneous sequence-specific identification and separation of **polynucleotide** fragments in a **polynucleotide** population (P1) comprises using restriction endonucleases that recognize degenerate bases in their recognition or cleavage sequence to produce P1 from transcribed **RNA**. The fragments are then **ligated** and amplified.

In detail, M1 comprises:

(a) reverse transcribing an **RNA** population to provide P1;

(b) digesting P1 with one or more restriction endonucleases (RE1) having a degenerate recognition or cleavage sequence comprising a degenerate base, where the degenerate base is represented by the formula of Nm, where N is the extent of degeneracy, and m is the number of degenerate bases, to produce restriction fragments having Nm different single-stranded overhangs for each restriction endonuclease;

(c) **ligating** the restriction fragments having the same overhangs to a series of adapters whose sequences are complementary to the overhangs; and

(d) amplifying the restriction fragments.

INDEPENDENT CLAIMS are included for the following:

(1) a method (M2) for detecting **polymorphism** comprising:

(a) reverse transcribing an **RNA** population to provide a **polynucleotide** population;

(b) digesting the **polynucleotide** population with one or

more RE1;

(c) **ligating** the restriction fragments having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(d) amplifying the restriction fragments;

(e) sequencing the amplified restriction fragments; and

(f) comparing the sequence of the amplified restriction fragments with the sequence of the same **polynucleotide** from a different source;

(2) a method (M3) for screening for interactions between a preselected protein and polypeptide fragments, comprising culturing the recombinant host cells of (1) under conditions which enable expression of correctly inserted restriction fragments by the host cell, and assaying the interaction of the polypeptide fragments encoded by the restriction fragments with the preselected protein;

(3) a method (M4) for detecting a change in the pattern of **RNA** expression in a tissue or cell associated with an internal or external factor comprising:

(a) determining the pattern of **RNA** expression in a first tissue or cell sample not subject to the internal or external change by a method comprising:

(i) reverse transcribing an **RNA** population to provide the **polynucleotide** population;

(ii) digesting a double-stranded cDNA library prepared from the **RNA** isolated from the first sample with one or more RE1, where m is 1-5;

(iii) **ligating** the restriction fragments produced from (ii) having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(iv) amplifying the restriction fragments; and

(v) displaying the pattern of **RNA** expression in the first sample;

(b) determining and optionally quantifying the pattern of **RNA** expression in a second tissue or cell sample subject to the physiological or pathological change by performing steps (a)-(d) of M1 with the second sample;

(c) digesting the restriction fragments obtained in step (b) with one or more further restriction endonucleases producing restriction fragments with single-stranded overhangs different from those produced in step (b); and

(d) comparing the first and the second displays to determine the effect of the internal or external factor on the pattern of **RNA** expression in the tissue;

(4) a method (M5) for diagnosis of a disease based on detecting a change in the pattern of **DNA** fragments in a disease tissue or cell sample comprising:

(a) determining the pattern of **DNA** fragments in the sample by:

(i) digesting a **DNA** isolated from the tissue or cell with one or more restriction RE1;

(ii) ligating the restriction fragments produced from (i) having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(iii) amplifying the restriction fragments; and

(iv) displaying the pattern of DNA fragments in the disease tissue or cell sample;

(b) determining the pattern of DNA fragments in a normal tissue or cell corresponding to the disease tissue by performing steps (a)-(d) of M1 with the normal tissue; and

(c) comparing the disease and the normal tissue or cell profile of DNA fragments;

(5) a method (M6) for detecting a change in the pattern of RNA

expression in a cell sample in response to an external factor comprising steps (a) to (d) of M4;

(6) a method (M7) for constructing an expressed sequence tag (EST) library comprising:

(a) reverse transcribing an mRNA population isolated from a eukaryotic source to provide a polynucleotide population;

(b) digesting a double-stranded cDNA library prepared from the mRNA with one or more RE1;

(c) ligating the restriction fragments produced from (b) having the same overhangs to a series of adapters whose sequences are complementary to the overhangs;

(d) amplifying the restriction fragments; and

(e) ligating the amplified restriction fragments into a suitable cloning vector;

(7) an isolated nucleic acid comprising an oligonucleotide selected from one of the 64 nucleotide sequences defined in the specification; and

(8) a recombinant host cell transformed with a vector comprising cloned polymerase chain reaction (PCR) obtained from M1.

USE - The method is useful for the simultaneous sequence-specific identification, separation and quantitative measurement of polynucleotide fragments in a polynucleotide population. The method is applicable in DNA fingerprinting, differential display of mRNA, mutation and polymorphism identification, drug screening, molecular taxonomy, and diagnosis of diseases such as heart disease, lung disease, kidney disease, neurodegenerative disease, liver disease, a disease of the reproductive system, or cancer.

The isolated nucleic acid molecule comprising an oligonucleotide selected from one of the 64 nucleotide sequences defined in the specification can be used in any of the methods described above (claimed).

ADVANTAGE - The method is rapid, quantitative and it determines the gene expression level without the requirement of sequence information.
Dwg.0/0

L34 ANSWER 6 OF 34 HCA COPYRIGHT 2002 ACS

136:49321 Methods for identifying osteolevin gene **polymorphisms** and for diagnosis, prevention and treatment of diseases associated with abnormal bone formation. Balemans, Wendy; Ebeling, Martin; Foernzler, Dorothee; Patel, Neela; Van Hul, Wim; Vickery, Brian Henry (F. Hoffmann-La Roche A.-G., Switz.; Universitaire Instelling Antwerpen (U.I.A.)). PCT Int. Appl. WO 2001098491 A2 20011227, 70 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CO, CU, CZ, DE, DK, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-EP6795 20010615. PRIORITY: EP 2000-112867 20000619.

AB The present invention relates generally to genetic **polymorphisms** in the Van Buchem-sclerosteosis disease region. In particular, the present invention relates to genetic **polymorphisms** in the Van Buchem-sclerosteosis disease region that are assocd. with disorders resulting in either net excess bone formation or insufficient bone formation in humans. Furthermore, isolated nucleic acid mols. encoding human osteolevin and genetic **polymorphisms** assocd. with disease are provided. Osteolevin polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. A genome-wide search with highly polymorphic microsatellite markers showed linkage of the disease locus to chromosome 17q12-21. Unraveling the underlying mechanism for this disorder could contribute to the understanding of the regulatory processes conditioning bone d. and the underlying pathol. processes.

Searched by: Mary Hale 308-4258 CM-1 1E01

L34 ANSWER 7 OF 34 HCA COPYRIGHT 2002 ACS
134:142722 Determination of sequence variations in nucleic acids by electrochemical detection of hybrids using probes labeled with redox groups. Umek, Robert M. (Clinical Micro Sensors, Inc., USA). PCT Int. Appl. WO 2001007665 A2 20010201, 158 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US20476 20000726. PRIORITY: US 1999-PV145695 19990726; US 2000-PV190259 20000317.

AB The present invention is directed to methods and compns. for the use of self-assembled monolayers to electronically detect nucleic acids, particularly alterations such as **nucleotide** substitutions (mismatches) and single **nucleotide polymorphisms** (SNPs). The method uses arrays of probes immobilized in self-assembling monolayers on an electrode surface. Probes and target sequences are labeled with redox groups and hybridization of the probe results in a change in redox potential. Prepn. of chips with probes immobilized via disulfide bridges to thiolated **DNA** is demonstrated and the effects of variables such as temp. on hybridization are studied. Methods of using competimers, perfectly matching probes that will replace weak or unstable hybrids, to improve the specificity of the hybridization are described.

L34 ANSWER 8 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2002-147802 [19] WPIDS
AB WO 200198527 A UPAB: 20020321

NOVELTY - Highly parallel characterization of **polymorphisms** by:

(a) binding a set of probes (P) to an addressable surface;

(b) hybridizing test nucleic acid (I) to P;

(c) extending P by allele-specific reaction, depending on sequence of (I) serving as template;

(d) treating with an exonuclease (II) that degrades unextended, but not extended, P; and

(e) analysis of remaining allele-specific extension products.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a kit comprising at least one primer pair for amplification;

(2) a set of (P);

(3) enzyme; and

(4) buffer and instructions for performing the new method.

USE - For highly parallel characterization of **polymorphisms** (claimed). Especially the method is useful for (i) genotyping known **polymorphisms**; (ii) identifying new **polymorphisms** and/or (iii) detecting and visualizing cytosine methylation patterns.

Specific applications are diagnosis and/or prognosis of: side effects of drugs; cancer; central nervous system disorders; aggression/behavioral disorders; brain damage; psychotic or personality disorders; cardiovascular, gastrointestinal or respiratory diseases; injury; inflammation; infection; convalescence; disorders of development, skin, muscle, connective tissue and bone; endocrine or metabolic disorders; headache and sexual dysfunction. It can also be used to differentiate between cell types and tissues, also for studying cell differentiation.

ADVANTAGE - The highly parallel method is more efficient than current processes as regards simplicity, cost, quality and throughput, and makes possible simultaneous detection of both cytosine methylation pattern and **polymorphisms**.

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Dwg.0/4

L34 ANSWER 9 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2002-075173 [10] WPIDS
AB WO 200183828 A UPAB: 20020213
NOVELTY - Diagnosing, (M1), genetic susceptibility to a disease, condition or disorder, (D), e.g. end stage renal disease or cancers of lung, breast or prostate, comprising obtaining a nucleic acid sample and detecting a single **nucleotide polymorphism**, (SNP), associated with a genetic predisposition for (D), in the transforming growth factor (TGF) beta -RII gene, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated **polynucleotide** (I) comprising at least 10 contiguous **nucleotides** of a sequence (S1) comprising 1883 **nucleotides** fully defined in the specification, or its complement, and containing at least one SNP at position 945, 983 or 1009 of S1;

(2) a kit (II) comprising (I), and instructions for using (I) for detecting the presence or absence of at least one SNP in the nucleic acid; and

(3) treatment (M2) or prophylaxis in a subject, which involves obtaining a sample of biological material containing nucleic acid from a subject, analyzing the nucleic acid to detect the presence of absence of at least one SNP in S1 or its complement, associated with (D), and treating (D).

ACTIVITY - Nephrotropic; cytostatic.

MECHANISM OF ACTION - Gene therapy. No supporting data given.

USE - M1 is useful for diagnosing genetic susceptibility for a disease, condition or disorder, e.g. end stage renal disease, lung cancer, breast cancer and prostate cancer (claimed). The method is useful for designing prophylactic treatment regimes for patients determined to have an increased susceptibility to the above disease, condition or disorders.
Dwg.0/11

L34 ANSWER 10 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2002-010807 [01] WPIDS
AB WO 200175175 A UPAB: 20020208
NOVELTY - Determining whether a subject has or is at risk of developing colorectal cancer comprises determining the identity of the allelic variant of the manganese superoxide dismutase (MnSOD) gene locus in a nucleic acid obtained from the subject.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a kit for determining whether a subject has or is at risk of developing a colorectal cancer, and for amplifying and/or determining the molecular structure of at least a portion of the MnSOD gene; and

(2) a method for determining risk of colorectal cancer in a subject comprising:

(a) determining the base identity of a portion of genomic **DNA** from the subject's cell sample, where the genomic **DNA** comprises an MnSOD gene comprising a mitochondrial targeting sequence, and the portion corresponding to position 351 of a fully defined sequence of 418 base pairs (I) given in the specification, of the MnSOD gene in the mitochondrial targeting sequence; and

(b) correlating the base identity with a risk for colorectal cancer.

USE - The method is useful for the early diagnosis of colorectal cancer by determining patient's susceptibility to the disease. The **polymorphism** of the manganese superoxide dismutase gene may be used to identify young individuals with high risk for cancer, and select them for further diagnostic procedures to maximize the benefits of treatment by detecting cancer in its early stages. Information obtained using the diagnostic method is useful for diagnosing or confirming that a

subject has an allele of a polymorphic region associated with colorectal cancer. Knowledge of the identity of a particular MnSOD allele in an individual, alone or in conjunction with information on other genetic defects contributing to the same disease allows customization of therapy for a particular disease to the individual's genetic profile. Identification of the allele of the MnSOD gene can also be useful for identifying an individual among other individuals from the same species, e.g. in fingerprinting. The nucleic acid or protein can be the basis for probes or primers, e.g., in methods for determining the identity of the allelic variant of the MnSOD polymorphic region, and can also be used to prepare MnSOD polypeptides for gene therapy or for preparing reagents to detect the MnSOD signal peptide or its allelic variant.

ADVANTAGE - The method is more efficient for early diagnosis and treatment of colorectal cancer. The method does not require the determination of large amounts of extraneous sequence data.
Dwg.0/2

L34 ANSWER 11 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-616537 [71] WPIDS
AB WO 200173130 A UPAB: 20011203
NOVELTY - Diagnosing (M1) a genetic susceptibility for a disease, condition or disorder in a subject, comprising analyzing the nucleic acid obtained from a biological sample to detect a single **nucleotide polymorphism** (SNP) in transforming growth factor- beta 1 (TGF-beta 1) gene, is new. SNP is associated with genetic susceptibility to a disease e.g. hypertension or end-stage renal disease due to hypertension.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated **polynucleotide** (I) comprising at least 10 contiguous **nucleotides** of a 2205 base pair sequence (S), fully defined in the specification or its complement, and containing at least one SNP at position 474, 510 or 546 of (S), where SNP is associated with end-stage renal disease (ESRD) due to hypertension, or containing at least one SNP at position 474, 510, 546 or 563 of (S), where SNP is associated with hypertension;

(2) a kit (II) comprising (I), and containing at least one SNP associated with hypertension or end-stage renal disease (ESRD) due to hypertension, and instructions for using (I) for detecting the presence or absence of SNP in the nucleic acid; and

(3) treatment or prophylaxis (M2) in a subject, comprising:

(a) obtaining a sample of biological material containing nucleic acid from a subject;

(b) analyzing the nucleic acid to detect the presence or absence of at least one SNP in (S) or its complement associated with hypertension or ESRD due to hypertension; and

(c) treating the subject for the disease, condition or disorder.

ACTIVITY - Hypotensive.

No biological data is given.

MECHANISM OF ACTION - None given.

USE - M1 is useful for detecting genetic susceptibility for a disease, condition, or disorder such as hypertension or end-stage renal disease (ESRD) due to hypertension in a subject. M2 is useful for prophylaxis or treatment of hypertension or ESRD due to hypertension in a subject. (All claimed).
Dwg.0/0

L34 ANSWER 12 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-616535 [71] WPIDS
AB WO 200173128 A UPAB: 20011203
NOVELTY - Diagnosing (M1) genetic susceptibility for disease or disorder in a subject involves analyzing nucleic acid obtained from biological sample from subject to detect single **nucleotide**

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polymorphism (SNP) in type II transforming growth factor- beta 1 receptor (TGF beta -RII) gene. SNP is associated with genetic predisposition to a disease such as hypertension or end-stage renal disease due to hypertension.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated **polynucleotide** (I) comprising at least 10 contiguous **nucleotides** of a sequence (S1) comprising 1883 base pairs fully defined in the specification or its complement, and containing at least one SNP at position 796, 820, 845, 876, 945 or 983 of S1, where SNP is associated with a disease such as hypertension or end-stage renal disease (ESRD) due to hypertension;

(2) a kit (II) comprising (I), and containing at least one SNP associated with a disease, condition or disorder such as hypertension or ESRD due to hypertension, and instructions for using (I) for detecting the presence or absence of SNP in the nucleic acid; and

(3) a method (M2) for treatment or prophylaxis in a subject involves obtaining a sample of biological material containing nucleic acid from a subject, analyzing the nucleic acid to detect the presence or absence of at least one SNP in S1 or its complement associated with a disease, condition or disorder such as hypertension or ESRD due to hypertension, and treating the subject for the disease, condition or disorder.

ACTIVITY - Hypotensive.

No supporting biological data is given.

MECHANISM OF ACTION - None given.

No supporting biological data is given.

USE - The method is useful for detecting genetic susceptibility for a disease, condition, or disorder such as hypertension or ESRD due to hypertension in a subject. M2 is useful for prophylaxis or treatment of hypertension or ESRD due to hypertension in a subject (claimed).

Dwg.0/0

L34 ANSWER 13 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-522607 [57] WPIDS
AB WO 200161043 A UPAB: 20011005

NOVELTY - Parallel genotyping (GT) of multiple patients comprises providing an array composition comprising a substrate (S) with a surface having discrete sites and a population of microspheres (MS) distributed on the surface, where MS comprise two subpopulations, each having several target analytes, contacting the array with a set of readout probes and detecting the presence of target analyte.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for an array composition comprising a (S) with a surface comprising discrete sites and a population of MS comprising two subpopulations, each comprising several target analytes, where the MS are distributed on the surface.

USE - The method is useful for parallel genotyping of multiple patients and for determining the identification of a **nucleotide** at the detection position in a target sequence (claimed). The array is useful for detecting and analyzing genotypes, single **nucleotide polymorphisms** and the presence or absence of subsequences such as genes in a sample for e.g. the presence or absence of chromosomal aberrations such as deletions or duplications in tumor samples. In addition, the method is useful for quantitation of the amount of target sequence.

ADVANTAGE - The method facilitates parallel analysis of a large number of sample components from a large number of patients.

Dwg.0/4

L34 ANSWER 14 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-451916 [48] WPIDS
CR 2002-188635 [15]

Searched by: Mary Hale 308-4258 CM-1 1E01

AB WO 200153537 A UPAB: 20020610
NOVELTY - Diagnosing a genetic predisposition to disease, condition or disorder i.e. hypertension, end stage renal disease due to hypertension, non insulin dependent diabetes mellitus and end stage renal disease due to non insulin dependent mellitus comprising detecting an associated **polymorphism** is new.

DETAILED DESCRIPTION - The diagnosis comprises obtaining a sample containing **DNA** and analyzing it to detect the presence or absence of a single **nucleotide polymorphism** in a nucleic acid sequence (I), a 3586 bp sequence given in a specification.

INDEPENDENT CLAIMS are included for the following:

(1) a kit comprising at least one **polynucleotide** of at least 10 **nucleotides** of (I) or its complement and containing at least one single **nucleotide polymorphism** associated with a disease disorder or condition of the invention or breast lung or prostate cancer as well as instructions for using the **polynucleotide** for detecting the presence or absence of the **polymorphism**; and

(2) the treatment or prophylaxis of a disorder or condition of the invention or breast lung or prostate cancer.

ACTIVITY - Nephrotropic.

MECHANISM OF ACTION - Vasodilatory. Antagonizer of vasoconstrictive effects of angiotensin II and endothelins. Angiotensin II promotes renal injury.

USE - The method is useful for diagnosing a genetic predisposition to disease, condition or disorder i.e. hypertension, end stage renal disease due to hypertension, non insulin dependent diabetes mellitus and end stage renal disease due to non insulin dependent mellitus, breast cancer, lung cancer and prostate cancer by identifying a **polymorphism** associated with the disorder.

ADVANTAGE - The method allows the identification people with a predisposition to disorders i.e. end stage renal disease and allow the identification and care of patients before the disease progresses to an end stage. Preventing a patient from developing end stage renal disease and therefore renal failure would save 30 000 US dollars per patient per year.

Dwg.0/0

L34 ANSWER 15 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-425680 [45] WPIDS
AB WO 200148244 A UPAB: 20010813
NOVELTY - Detecting (I) a result from identification reaction to identify selected **nucleotide**(s) in target nucleic acid(s) (TNA), involves using target **oligonucleotide**(s) having a first complementarity region (CR1), complementary to a region of TNA and a second complementarity region (CR2) located 5' of CR1, complementary to a capture **oligonucleotide** (CON) linked to a mobile solid support.

DETAILED DESCRIPTION - (I), comprises:

(a) contacting a target **oligonucleotide**(s) comprising CR1 and CR2, where CR2 is 5' of CR1 and CR1 comprises a region complementary to a section of TNA that is directly 3' of and adjacent to the selected **nucleotide**(s), with a sample comprising TNA under hybridization conditions that allow the formation of a first hybridization product(s);

(b) performing, in the presence of a selectively labeled reporter probe(s), a selected identification with the first hybridization product(s) to determine the identity of the selected **nucleotide**(s), where a selectively labeled detection product(s) comprising the target **oligonucleotide**(s) and the reporter probe(s) can be formed;

(c) isolating the detection product(s) by contacting the detection product(s) with CON covalently coupled directly or indirectly to tagged mobile solid support(s), under hybridization conditions to form a second

hybridization product(s); and

(d) **detecting** the **label**(s) of the labeled detection product(s) in the second hybridization product(s) and detectable tags of the support in the same second hybridization product(s), where the presence of the label(s) and specific detectable tag indicates the identity of the selected **nucleotide**(s) in TNA.

USE - The method is useful for determining selected **nucleotide polymorphisms** in genomic **DNA**.

The method involves:

(i) performing an amplification of the genomic **DNA** using a first nucleic acid primer comprising a region complementary to a section of one strand of the nucleic acid that is 5' of the selected **nucleotide**, and a second nucleic acid primer complementary to a section of the opposite strand of nucleic acid downstream of the selected **nucleotide**, under conditions for specific amplification of the region of the selected nulceotide between the two primers, to form a PCR product;

(ii) performing an amplification of the genomic **DNA** using as a primer an **oligonucleotide** comprising a first region having a T7 **RNA** polymerase promoter and a second region complementary to a section of one strand of the nucleic acid that is directly 5' of the selected **nucleotide**, and using T7 **RNA** polymerase to amplify one strand into cRNA and using reverse transcriptase to amplify the second strand complementary to the cRNA strand, under conditions for specific amplification of the region of the nulceotide between the two primers, to form a cRNA amplification product; or

(iii) treating genomic **DNA** to decrease viscosity; and contacting sample comprising one or more PCR products, cRNA amplification products or treated genomic **DNA** with specific target **oligonucleotides** as described in (I), where identity of a selected **nucleotide** in the PCR, cRNA products or treated genomic **DNA** is identified.

The identities of the identified **nucleotides** is compared with a non-polymorphic **nucleotide**, where a different identity of the identified **nucleotide** from that of the non-polymorphic **nucleotide** indicates one or more **polymorphisms** in the genomic **DNA**.

(I) is also useful for cleavase/signal release reaction, polymerase/repair reaction to identify selected **nucleotides** in TNA and detecting selected microbial contaminants, preferably Staphylococcus aureus, Burkholdomonas cepacia, Escherichia coli or Pseudomonas in a sample. The CR1 complementary to a section of a nucleic acid that is specific to S.aureus has the sequence: gccggtggagtaacctttttag or gccggtggagtaacctttttagg, B. cepacia has the sequence: ctgagaggcgggagtgct or ctgagaggcgggagtgctc, E. coli or Pseudomonas has the sequence: aataccgcata and aataccgcatan, where n is c or a and Pseudomonas or B. cepacia has the sequence aataccgcatacg and aataccgcatacgn, where n is t or a (all claimed).

ADVANTAGE - A reduced number of bead sets are needed to analyze many different single **nucleotide polymorphisms**.

Dwg.0/2

L34 ANSWER 16 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-381715 [40] WPIDS
AB WO 200142512 A UPAB: 20010719

NOVELTY - Hybridization assay, involves contacting distinct sample **polynucleotide** target (T1) and a standard **polynucleotide** target (T2) with a detectable sample probe (P1) specific for T1, and an independently detectable standard probe (P2) specific for T2, under hybridization conditions, and detecting the hybridization pattern of the probes.

DETAILED DESCRIPTION - Hybridization assay, involves contacting

separate samples, each comprising T1 and T2, with P1 specific for T1 and P2 specific for T2, under hybridization conditions to produce a hybridization pattern comprising P1 signal and P2 signal at each sample, where P2 signal provides normalization control for the sample, and detecting the hybridization pattern.

INDEPENDENT CLAIMS are also included for the following:

(1) immobilizing (I) a **polynucleotide** probe, by combining the probe with a **polynucleotide** target stably associated with a surface of a solid support, where one of the probe and target is double-stranded comprising complementary strands, and the other is single-stranded having complementarity with one of the complementary strands, under conditions where the probe and target hybridize and the probe is thus immobilized; or combining the probe with a **polynucleotide** target stably associated with a surface of a solid support, where the probe and target are double-stranded comprising complementary strands and have complementarity, under conditions where the probe and target hybridize and the probe is immobilized;

(2) immobilizing and detecting (ID) a **polynucleotide** probe, by contacting a **polynucleotide** target which is stably associated with a surface of a solid support, with a double-stranded **polynucleotide** probe, and detecting specific hybridization of the probe to the target;

(3) a microarray (M) comprising a number of elements, where each element comprises a distinct sample **polynucleotide** target and a standard **polynucleotide** target; and

(4) a kit (K) for use in measuring hybridization, comprising (I) or a sample of T1 and T2, and P2.

USE - The method is useful for obtaining quantitative information about the genetic profile of a labeled nucleic acid sample, normalizing and quantitating hybridization reactions, for obtaining quantitative information about the physiological source of the sample nucleic acid, for comparing nucleic acid samples from two or more physiological sources to identify and quantify differences between the patterns, and thus providing data on the differential expression of a particular gene in the physiological sources being compared, in differential gene expression assays for the analysis of a diseased and normal tissue, analysis of a different tissue or subtissue types, and large scale correlation studies on the sequences, mutations, variants or **polymorphisms** among samples.

ADVANTAGE - The method provides quantitative information about each element of the microarray. Hybridization of the probe sequences and the standard sequences is not competitive, thereby reducing noise in the results.

Dwg.0/7

L34 ANSWER 17 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-327241 [34] WPIDS
AB US 6221603 B UPAB: 20010620

NOVELTY - New padlock probes, each comprising a nucleic acid sequence including 2 target hybridization end regions on opposing ends of each padlock probe, and a stuffer region located between the target hybridization end regions.

DETAILED DESCRIPTION - New padlock probes, each comprising a nucleic acid sequence including 2 target hybridization end regions on opposing ends of each padlock probe, where the target hybridization end regions are complementary to a target hybridization region on a single strand target nucleic acid sequence and may hybridize to the single strand target nucleic acid sequence to form a probe/target duplex and a stuffer region located between the target hybridization end regions.

An INDEPENDENT CLAIM is also included for a method for high throughput analysis of genetic loci comprising:

(a) providing a target nucleic acid sample;

(b) combining the target nucleic acid sample with padlock probes to form padlock probe/target hybrids;

(c) mixing ligase with the padlock probe/target nucleic acid duplex to form a **ligation** mixture, and incubating the **ligation** mixture under conditions promoting **ligation** of the padlock probes to form amplification target circles;

(d) mixing a primer which is complementary to the primer complementary sequence with the amplification target circles, and incubating the primer/amplification target circles mixture under conditions promoting hybridization between the amplification target circles and the primers;

(e) mixing **DNA** polymerase with the primer/amplification target circles mixture to produce a rolling circle replication mixture, and incubating the rolling circle amplification mixture under conditions promoting rolling circle amplification target circles and the primers, resulting in the formation of tandem sequence **DNA**;

(f) cutting each tandem sequence at the generated restriction enzyme site into sets of non-tandem **DNA** fragments of a discrete length, where each amplification target circle produces tandem sequence **DNA** that is cut into sets of non-tandem **DNA** fragments of a unique discrete size;

(g) separating by length the sets of non-tandem **DNA** fragments; and

(h) detecting the sets of separated non-tandem **DNA** fragments.

USE - The probes are useful in assaying target nucleic acid sequences, such as genomic **DNA**, plasmids, viral genomic **DNA** or **RNA**, cDNA or mRNA. The probes and the method are especially useful in high throughput analysis of different genetic loci at the same time to determine the presence or expression of a gene of interest and to detect a polymorphic variation at a specific site.

Dwg.0/6

L34 ANSWER 18 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-657558 [76] WPIDS
CR 2001-425661 [44]; 2002-010801 [67]
AB DE 10016348 A UPAB: 20020105

NOVELTY - Parallel sequencing of at least two different nucleic acids (NA), present in a mixture, is new.

DETAILED DESCRIPTION - Parallel sequencing of at least two different nucleic acids (NA), present in a mixture. At least one pair of primers is immobilized irreversibly on a surface and treated with an NA mixture containing molecules that can hybridize to both primers. The immobilized primers are extended, in complementary fashion, to form a counter strand, resulting in formation of secondary NA (sNA). The surface is freed of NA that is not irreversibly bound and the sNA amplified to form tertiary nucleic acid (tNA). Counter-strands (gtNA) of tNA are prepared and extended by a single **nucleotide** (nt) in which the 2'- or 3'-hydroxy is protected, and which is **detectably labeled**. The incorporated nt is identified, the protecting group removed and the label either removed or altered. The single-nt extension procedure is repeated until the required sequence information has been obtained.

INDEPENDENT CLAIMS are also included for the following:

(1) similar method in which tNA is treated so that it is bound to the surface only through the 5'-end of one strand, then cut with a type IIS restriction enzyme (RE) to generate 3' or 5' overhangs, determining one or more bases in these overhangs, **ligating** linkers to the free ends (these linkers include a recognition site for type IIS RE), treating again with RE that recognizes the site introduced in the linker and repeating the process as required;

(2) apparatus for performing the new process;

(3) method for localized amplification of NA, comprising the new

process as far as amplification to produce tNA; and

(4) surface-bound library of NA produced by the method for localized amplification of NA.

USE - The method is useful for detecting genes and transcripts (e.g. for expression analysis), identifying mutations and **polymorphisms**, and detecting organisms and viruses.

ADVANTAGE - The method provides highly parallel sequencing, requires relatively small amounts of **DNA**, can sequence long segments and does not require complex apparatus.

Dwg.0/13

L34 ANSWER 19 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2001:385141 Document No.: PREV200100385141. Assessment of the **nucleotide** sequence variability in the bovine T-cell receptor alpha delta joining gene region. Fries, R. (1); Ewald, D.; Thaller, G.; Buitkamp, J.. (1) Lehrstuhl fuer Tierzucht, Technische Universitaet Muenchen, Alte Akademie 12, 85350, Freising: Ruedi.Fries@tierzucht.tum.de Germany. Animal Biotechnology, (May, 2001) Vol. 12, No. 1, pp. 29-49. print. ISSN: 1049-5398. Language: English. Summary Language: English.

AB The sequence of 2193 **nucleotides** from the bovine T-cell receptor alpha / delta joining gene region (TCRADJ) was determined and compared with the corresponding human and murine sequences. The identity was 75.3% for the comparison of the Bos taurus vs. the Homo sapiens sequence and 63.8% for the Bos taurus vs. the Mus musculus sequence. This comparison permitted the identification of the putatively functional elements within the bovine sequence. Direct sequencing of 2110 **nucleotides** in nine animals revealed 12 variable sites. Estimates, based on direct sequencing in three Holstein Friesian animals, for the two measures of sequence variability, **nucleotide polymorphism** (u) and **nucleotide** diversity (p), were 0.00050 (60.00036) and 0.00077 (60.00056), respectively. The test statistic, Tajima's D, for the comparison of the two measures indicates that the difference between u and p is close to significance ($P<0.05$), suggesting the possibility of selective forces acting on the studied genomic region. Allelic variation at 5 of the 12 variable sites was analysed in 359 animals (48 Anatolian Black, 56 Braunvieh, 115 Fleckvieh, 47 Holstein Friesian, 50 Simmental and 43 Pinzgauer) using the **oligonucleotide ligation** assay (OLA) in combination with the enzyme linked immunoabsorbant assay (ELISA). Nine unambiguous haplotypes could be derived based on animals with a maximum of one heterozygous site. Four to seven haplotypes were present in the different breeds. When taking into account the frequencies of the haplotypes in the different breeds, especially in Anatolian Black, an ancestral cattle population, we could establish the likely phylogenetic relationships of the haplotypes. Such haplotype trees are the basis for cladistic candidate gene analysis. Our study demonstrates that the systematic search of single **nucleotide polymorphisms** (SNPs) is useful for analysing all aspects of variability of a given genomic region.

L34 ANSWER 20 OF 34 HCA COPYRIGHT 2002 ACS

133:262262 Detection of nucleic acid **polymorphisms** using the ligase detection reaction with addressable arrays of capture probes. Barany, Francis; Gerry, Norman P.; Witowski, Nancy E.; Day, Joseph; Hammer, Robert P.; Barany, George (Cornell Research Foundation, Inc., USA; Regents of the University of Minnesota; Board of Supervisors of Louisiana State University and Agricultural and Mech). PCT Int. Appl. WO 2000056927 A2 20000928, 217 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US7006 20000317. PRIORITY: US 1999-PV125357 19990319.

AB The present invention describes a method for identifying one or more of a plurality of sequences differing by one or more single base changes,

Searched by: Mary Hale 308-4258 CM-1 1E01

insertions, deletions, or translocations in a plurality of target **nucleotide** sequences. The **ligation** phase utilizes a **ligation** detection reaction between one **oligonucleotide** probe which has a target sequence-specific portion and an addressable array-specific portion, and a second **oligonucleotide** probe, having a target sequence specific portion and a **detectable label**. After the **ligation** phase, the capture phase is carried out by hybridizing the **ligated oligonucleotide** probes to a solid support with an array of immobilized capture **oligonucleotides** at least some of which are complementary to the addressable array-specific portion. Following completion of the capture phase, a detection phase is carried out to **detect** the **labels** of **ligated oligonucleotide** probes hybridized to the solid support. The **ligation** phase can be preceded by an amplification process. The present invention also relates to a kit for practicing this method, a method of forming arrays on solid supports, and the supports themselves.

L34 ANSWER 21 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-025173 [03] WPIDS
CR 2001-032066 [01]
AB WO 200071753 A UPAB: 20020513
NOVELTY - Determining (I) whether a subject has or is predisposed to developing an arterial restenosis, comprises detecting a restenosis associated allele (RAA) in a nucleic acid sample from the subject, where detection of the restenosis allele indicates that the subject has or is predisposed to the development of a restenosis.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a kit (II) for determining the existence of or a susceptibility to developing a restenosis in a subject, comprising a first primer **oligonucleotide** that hybridizes 5' or 3' to an allele selected from allele 1 of any of the following markers: interleukin-1 alpha (IL-1A) (+4845), interleukin-1 beta (IL-1B) (-511), IL-1B (+3954), interleukin-1 receptor antagonist (IL-1RN) (VNTR (variable number tandem repeat)) and IL-IRN (+2018) or an allele in linkage disequilibrium with it;

(2) selecting (III) an appropriate therapeutic for an individual that has or is predisposed to developing a restenosis, by detecting whether the subject contains a RAA and selecting a therapeutic that compensates for a restenosis causative functional mutation that is in linkage disequilibrium with the RAA;

(3) determining (IV) the effectiveness of treating a subject that has or is predisposed to developing restenosis with a particular dose of a restenosis therapeutic, comprising:

(a) detecting the level, amount or activity of an IL-1 protein, or an IL-1 mRNA or **DNA** in a sample obtained from a subject;

(b) administering the particular dose of the particular therapeutic to the subject, detecting the level, amount or activity of an IL-1 protein; or an IL-1 mRNA or **DNA** in a sample obtained from a subject; and

(c) comparing the relative level, amount or activity obtained in step (a) with the level, amount or activity obtained in step (b);

(4) treating (V) or preventing the development of a restenosis in a subject comprising the steps of detecting the presence of a RAA and administering to the subject a therapeutic that compensates for causative mutation that is in linkage disequilibrium with the RAA;

(5) screening (VI) for a restenosis therapeutic, comprising:

(a) combining an IL-1 polypeptide or its bioactive fragment, an IL-1 binding partner and a test compound under conditions where, but for the test compound, the IL-1 protein and IL-1 binding partner are able to interact; and

(b) detecting the extend to which, in the presence of the test

compound, an IL-1 protein/IL-1 binding partner complex is formed, where an increase in the amount of complex formed by an agonist in the presence of the compound relative to in the absence of the compound or a decrease in the amount of complex formed by an antagonist in the presence of the compound relative to in the absence of the compound indicates that the compound is a restenosis therapeutic; and

(6) identifying (VLI) a restenosis therapeutic, comprising:

(a) contacting an appropriate amount of a candidate compound with a cell or cellular extract, which expresses an IL-1 gene; and

(b) determining the resulting protein bioactivity, where a decrease of an agonist or an antagonist bioactivity in the presence of the compound as compared to the bioactivity in the absence of the compound indicates that the candidate is a restenosis therapeutic.

ACTIVITY - Vasotropic; antiinflammatory; hypotensive; anticoagulant; antilipemic. No supporting data is given.

MECHANISM OF ACTION - Modulators of interleukin-1 alpha and beta and interleukin-1 receptor antagonist activity.

USE - The method is useful for determining whether a subject has or is predisposed to developing an arterial restenosis (claimed). The restenosis associated allelic pattern permits the diagnosis of occlusive cardiovascular disorder. The diagnosis allows the most suitable treatment methods for restenosis to be used e.g. selecting therapies for initial vascular stenosis most likely to avoid subsequent stenoses.

The detection methods identify restenosis therapeutics, agonists and antagonists, (proteins, peptides, peptidomimetics, small molecules or nucleic acids e.g. anti-sense, ribozyme and triplex nucleic acids) which are used to treat restenosis.

Dwg.0/10

L34 ANSWER 22 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2001-007020 [01] WPIDS
AB WO 200063437 A UPAB: 20001230

NOVELTY - Microsphere arrays are used to detect and quantify a number of nucleic acid reactions.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) identifying a **nucleotide** at a detection position (DP) in a target sequence (TS) comprising:

(a) providing a hybridization complex (HC) comprising the TS and a capture probe (CP) attached to a microsphere on a surface of a patterned substrate; and

(b) determining the **nucleotide** at the DP;

(2) sequencing a target nucleic acid (NA) with two adjacent domains (with target positions), comprising providing a HC comprising the TS and a CP attached to a microsphere on a surface of a patterned substrate and determining the identity of bases at the target positions;

(3) identifying a **nucleotide** at a DP in a TS with a target domain (TD) containing the DP and a second TD adjacent to the DP, comprising:

(a) hybridizing a first **ligation** probe to the first TD;

(b) hybridizing a second **ligation** probe to the second TD, so that, if the second **ligation** probe comprises a base that is perfectly complementary to the DP, a **ligation** structure is formed;

(c) **ligating** the probes to form a **ligated** probe using an enzyme;

(d) forming an assay complex with:

(i) the **ligated** probe;

(ii) a CP covalently attached to a microsphere on a surface of a substrate; and

(iii) a label;

(e) detecting the presence or absence of the label as an indication

of the formation of the **ligation** structure; and

(f) identifying the base at the DP

(4) sequencing target NA's, containing adjacent domains (with target positions), comprising:

(a) providing HCs attached to a surface of a substrate, each HC comprising a TS and a sequencing primer that hybridizes to the first domain of the TS;

(b) extending the primers by the addition of a **nucleotide** to the DP using an enzyme to form an extended primer; and

(c) detecting the release of pyrophosphate to determine the type of the first **nucleotide** added onto the primers;

(5) detecting a target NA sequence, comprising:

(a) attaching an adapter NA to a target NA sequence to form a modified target NA sequence;

(b) contacting the modified target NA sequence with an array containing:

(i) a substrate with a patterned surface and discrete sites; and

(ii) a population of microspheres (distributed on the surface) with a subpopulation of CP (which forms a HC with the modified target NA sequence); and

(c) detecting the presence of the modified target NA sequence;

(6) detecting a target NA sequence, comprising:

(a) hybridizing a primer (with an adapter sequence) to a portion of a TS;

(b) hybridizing a second primer to a second portion of the TS;

(c) ligating the first and second primers together to form a modified primer;

(d) contacting the adapter sequence of the modified primer with an array containing:

(i) a substrate with a surface comprising discrete sites; and

(ii) a population of microspheres (distributed on the surface) with a subpopulation comprising a first CP (which forms a HC with the modified primer); and

(e) detecting the presence of the modified primer;

(7) detecting a target NA sequence, comprising:

(a) hybridizing a primer NA to the TS to form a first HC;

(b) contacting the HC with an enzyme to form a modified primer NA;

(c) disassociating the HC;

(d) contacting the modified primer NA with an array containing:

(i) a substrate with a surface of discrete sites; and

(ii) a population of microspheres (distributed on the surface) comprising a subpopulation with a CP (which forms an assay complex with the modified primer); and

(e) detecting the presence of the modified primer NA;

(8) detecting a target NA, comprising:

(a) hybridizing a primer to a TS to form a HC;

(b) contacting the HC with an enzyme to extend the primer to form a newly synthesized strand and an NA hybrid that comprises an RNA polymerase promoter;

(c) contacting the hybrid with an RNA polymerase that recognizes the RNA polymerase promoter and generates a newly synthesized RNA strand;

(d) contacting the newly synthesized RNA strand with an array comprising:

(i) a substrate with a surface of discrete sites; and

(ii) a population of microspheres (distributed on the surface) containing a subpopulation with a CP (which forms an assay complex with the modified primer); and

(e) detecting the presence of the newly synthesized RNA strand;

(9) a kit for NA sequencing comprising:

(a) a composition of:

(i) a substrate with a patterned surface and discrete sites; and

(ii) a population of microspheres (which comprise CPs) distributed on

the sites;
(b) an extension enzyme; and
(c) dNTPs; and
(10) a kit for detection of a target NA sequence comprising:
(a) a NA primer complementary to a domain of the TS;
(b) an enzyme that will modify the NA primer; and
(c) an array containing:
(i) a substrate with a patterned surface of discrete sites; and
(ii) a population of microspheres (distributed on the surface) comprising first and second subpopulations which each contain a bioactive agent.

ACTIVITY - Antibacterial; antiviral; anticancer. No biological data is given.

MECHANISM OF ACTION - None given.

USE - The processes are useful for determining the identity of nucleotides at detection positions in a target sequence. They may be used in genotyping, e.g., for determining alterations such as nucleotide substitutions (mismatches) and single nucleotide polymorphisms, and in NA sequencing. They may be used for identifying infectious organisms such as bacteria or viruses, for identifying mutant genes such as oncogenes, in tissue typing for compatibility preceding tissue transplantation, in matching tissue or blood samples for forensic medicine or for exploring homology among genes from different species.

ADVANTAGE - Using fiber optic technology, extremely high density arrays can be made. Beads of 200 micro m or less can be used in the microsphere arrays with beads of 200 nm being possible. As very small fibers are known, it is possible to have as many as 40,000 or more different elements (e.g. fibers and beads) in a 1 mm2 fiber optic bundle, with densities of greater than 25,000,000 individual beads and fibers per 0.5 cm2 obtainable.

Dwg.0/17

L34 ANSWER 23 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2000-611449 [58] WPIDS
AB WO 200053812 A UPAB: 20001114

NOVELTY - Making (M1) immobilized nucleic acid molecule array (N) comprises creating array of spots of nucleic acid capture activity (I) contacting (I) with excess of (N) with an excluded column diameter greater than the diameter of the spots of (I), resulting in (N), in which each spot of (I) can bind only (N) with excluded volume diameter greater than size of spots of (I).

DETAILED DESCRIPTION - The spots of the capture activity are separated by a distance greater than diameter of the spots and size of the spots is less than the diameter of the excluded volume of nucleic acid molecule to be captured.

INDEPENDENT CLAIMS are also included for the following:

(1) detecting (M2) a nucleic acid on (N) comprising generating multiple (N) in which the nucleic acid molecules of each unit of (N) occupy positions which corresponds to those positions occupied by the nucleic acid molecules of each unit of the multiple (N) array and then subjecting one or more units of the multiple (N) (but at least one less than the total number of the multiple (N)) to a method of signal detection which involves a signal amplification method that renders each member of the multiple nucleic acid array non-reusable;

(2) preserving (M3) the resolution of nucleic acid features on a first immobilized array during cycles of array replication involves amplifying the features of a first array to yield an array of features with a hemisphere radius (r) and a cross-sectional area (q) at the surface supporting the array, such that the features remain essential distinct;

(3) making (M4) multiple (N) comprising:

(a) providing a first liquid mixture of template nucleic acid, one **oligonucleotide** primer, which includes a linker moiety, and

monomers capable of forming a polymerized gel matrix;

(b) contacting the mixture with a solid support;

(c) forming a first layer of a polymerized gel matrix with the linker moiety covalently bound to it;

(d) providing a second liquid mixture of one **oligonucleotide** primer and monomers capable of forming a polymerized gel matrix;

(e) contacting the first layer with the second liquid matrix;

(f) forming a second layer of a polymerized gel matrix;

(g) amplifying the template nucleic acid and transferring amplified nucleic acid to the second layer;

(h) removing the second layer; and

(i) optionally repeating steps (c) - (g);

(4) determining (M5) the **nucleotide** sequence of an immobilized nucleic acid array comprising:

(a) **ligating** a first double-stranded nucleic acid probe having a restriction endonuclease recognition site which is separate from the cleavage site, to one end of a nucleic acid of the array;

(b) identifying one or more **nucleotides** at the end of the **polynucleotide** by the identity of the first double stranded nucleic acid probe **ligated** to it or by extending a strand of the **polynucleotide** or probe;

(c) amplifying the features of the array using a primer complementary to the first double stranded nucleic acid probe, such that only molecules which have been successfully **ligated** with the first double stranded nucleic acid probe are amplified;

(d) contacting the amplified array with support such that a subset of nucleic acid molecules produced by the amplifying are transferred to the support;

(e) covalently attaching the subset of nucleic acid molecules transferred in the above step to the support to form a replica of the amplified array;

(f) cleaving the nucleic acid features of the array with a nuclease recognizing the nuclease recognition site of the probe such that the nucleic acid of the features is shortened by one or more **nucleotides**; and

(g) repeating steps (a) - (f) until the **nucleotide** sequences of the features of the array are determined;

(5) a method (M6) of determining the **nucleotide** sequence of the features of (N) comprising:

(a) adding a mixture comprising an **oligonucleotide** primer and a template-dependent polymerase to an array of immobilized nucleic acid features;

(b) adding a single, fluorescently labeled deoxynucleoside triphosphate to the mixture;

(c) **detecting** incorporated **label** by monitoring fluorescence;

(d) repeating steps (b) and (c) with each of the remaining three labeled deoxynucleoside triphosphates in turn; and

(e) repeating steps (b) - (d) until the **nucleotide** sequence is determined;

(6) a method (M7) of determining the nucleotide sequence of the features of micro-array of nucleic acid comprising:

(a) creating a micro-array of nucleic acid features in a linear arrangement within and along one side of a polyacrylamide gel, the gel further comprising one or more oligonucleotide primers and a template-dependent polymerizing activity;

(b) amplifying the micro-array of (a);

(c) adding a mixture of deoxynucleoside triphosphates, comprising:

(i) each of the four deoxynucleoside triphosphates; and

(ii) chain-terminating analogs of each of the deoxynucleoside triphosphates labeled with a spectrally distinguishable fluorescent moiety;

(d) incubating the mixture with the micro-array;

(e) electrophoretically separating the products of the extension within the polyacrylamide gel; and

(f) determining the nucleotide sequence of the features of the micro-array by detecting the fluorescence of the extended, terminated and separated reaction products within the gel; and

(7) a method (M8) for simultaneously amplifying multiple nucleic acids comprising:

(a) creating a micro-array of immobilized oligonucleotide primers;

(b) incubating the micro-array of step (a) with amplification template and a non-immobilized oligonucleotide primer;

(c) incubating the hybridized primers and template with a DNA polymerase and deoxynucleotide triphosphates; and

(d) repeating steps (b) and (c) for a defined number of cycles to yield multiple amplified DNA molecules.

USE - For nucleic acid replication or amplification, genomic characterization, gene expression studies, medical diagnostics e.g. expression analysis and genetic polymorphism detection. They are also of use in DNA/protein binding assays and more general protein array binding assays. The methods are also useful for determining the sequences of nucleic acid on arrays.

ADVANTAGE - By using the novel nucleic acid arrays a full genome including unknown DNA sequences can be replicated. The size of the nucleic acid fragments or primers to be replicated can be from about 25-mer to about 9000-mer. The method is also quick and cost effective. The thickness of the chip is 3000 nm which provides a much higher sensitivity. The chips are compatible with inexpensive in situ polymerase chain reaction (PCR) devices, and can be reused as many as 100 times.
Dwg.0/10

L34 ANSWER 24 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2000-549279 [50] WPIDS
AB WO 200049169 A UPAB: 20001010
NOVELTY - Diagnosing a genetic susceptibility for end-stage renal disease (ESRD) in a subject involves obtaining a biological sample comprising at least one **polynucleotide** from the subject and analyzing the **polynucleotide** to detect genetic **polymorphism** associated with an altered susceptibility for ESRD.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated nucleic acid sequence (I) comprising at least 10 contiguous **nucleotides** of a sequence (S1) comprising 2195 or 1883 **nucleotides** (as given in the specification) and with at least a variant associated with ESRD so that (I) differs from S1; and

(2) a kit (K) for detecting **polymorphism** comprising (I), a unit for detecting the **polymorphism** and instructions for use.

ACTIVITY - Renal; antidiabetic.
No data given.

MECHANISM OF ACTION - Gene therapy.

USE - The method is useful for diagnosing and treating, preferably treating prophylactically, a **polymorphism** associated with ESRD associated with hypertension (claimed) or non-insulin dependent diabetes mellitus. The treatment counteracts the effect of **polymorphism**.

ADVANTAGE - The method paves way for early detection of ESRD and hence effective delay or ideally, prevention of ESRD is made possible.
Dwg.0/2

L34 ANSWER 25 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2000-465999 [40] WPIDS
AB WO 200040755 A UPAB: 20000823
NOVELTY - A new method for assembling genomic maps of an organisms **DNA** comprises creating representations of the genome from the

organisms **DNA** library and generating nucleic acid sequence information from these representations. Clone overlap and sequence information from different representations is then combined to assemble a genomic map of the organism.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of identifying single **nucleotide polymorphisms** (SNP) in genomic **DNA** comprising:

(a) creating representations of the genomes of multiple individuals;

(b) creating a representational library from the representation;

(c) generating nucleic acid sequence information from individual clones of the representational library; and

(d) analyzing the sequence information to identify single **nucleotide polymorphisms** among the multiple individuals.

(2) a method for large scale detection of single **nucleotide polymorphisms** on a **DNA** array; and

(3) a method to sequence directly from a polymerase chain reaction (PCR) amplified nucleic acid without primer interference comprising:

(a) amplifying the nucleic acid with primers containing alternative nucleoside bases under conditions effective to produce amplification products;

(b) cleaving both incorporated and unincorporated primers under conditions which leave the amplification products intact.

USE - The method is useful for identifying SNP in genomic **DNA** or on a **DNA** array. The method of (2) is used to quantify an allele imbalance between first and second alleles. In particularly, this method is useful for quantifying LOH or gene amplification in a tumor sample containing up to 50% stromal contamination.

ADVANTAGE - The method reduces the need for multiple primers, therefore giving significant savings in time and cost of a large-scale SNP analysis.

Dwg.0/75

L34 ANSWER 26 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 2000-136988 [12] WPIDS
AB WO 9967414 A UPAB: 20000308

NOVELTY - A method for identifying a selected **nucleotide** in a nucleic acid uses a mobile solid support for either a primer, one component of a **ligation** reaction or a capture **oligonucleotide**.

DETAILED DESCRIPTION - The nucleic acid is hybridized with a primer that

(a) is linked at its 5' end to a detectably tagged mobile solid support; and

(b) includes a region complementary to a section of directly 3' of, and adjacent to, selected nt.

A primer extension reaction is performed to incorporate a **detectably labeled**, chain-terminating **nucleotide** and the presence or absence of the label in the hybridization product is determined. The presence and identity of the label indicates which chain-terminating **nucleotide** has been incorporated and the identity of the complementary to the selected **nucleotide**, and identifies the selected **nucleotide** in a nucleic acid.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of determining a selected **nucleotide polymorphism** in genomic **DNA** treated to reduce viscosity, comprising;

(a) amplifying the genomic **DNA** using a polymerase chain reaction (PCR);

(b) contacting the PCR product with a nucleic acid linked at its 5' end to a detectably tagged mobile solid support, the nucleic acid comprises a region complementary to a section of one strand of the PCR

product which is directly 5' to the selected **nucleotide**, under hybridization conditions;

(c) performing a primer extention reaction with the hybridization product and a **detectably labeled**, identified chain-terminating **nucleotide**;

(d) detecting the presence or absence of a label incorporated into the hybridization product, indicating the identity of the **nucleotide** complementary to the selected **nucleotide**; and

(e) comparing the identity of the selected **nucleotide** with a non-polymorphic **nucleotide**, a different identity indicates a **polymorphism** of that selected **nucleotide**;

(2) a method for determining a selected **nucleotide polymorphism** in genomic **DNA** treated to reduce viscosity, comprising the method of (1) where step (a) uses as a primer an **oligonucleotide** comprising a first region having a T7 **RNA** polymerase promoter and a second region complementary to a section of one strand of the nucleic acid which is directly 5' of the selected **nucleotide**, and **RNA** polymerase to amplify one strand into cRNA and reverse transcriptase to amplify a second strand complementary to the first, and amplifying to form an amplification product;

(3) a method of determining a selected **nucleotide polymorphism** in genomic **DNA** treated to reduce viscosity, comprising

(a) contacting the genomic **DNA** with a first primer linked at its 5' end to a detectably tagged mobile solid support, the primer comprises a region complementary to a section of one strand of the genomic **DNA** directly 5' of and adjacent to the selected **nucleotide** ; and

(b) performing steps (c), (d) and (e) of method (1);

(4) a method of identifying a selected **nucleotide** in a first nucleic acid comprising:

(a) contacting the first nucleic acid with a second nucleic acid, linked at its 5' end to a detectably tagged mobile solid support, which comprises a region complementary to a section directly 3' of the selected **nucleotide**, and terminates at its 3' end in a test **nucleotide** positioned to base-pair with the selected **nucleotide**, and a third, fluorescently labeled nucleic acid, which comprises a region complementary to a section directly 5' of and adjacent to the selected **nucleotide**;

(b) adding to the hybridization product a ligase; and

(c) detecting the presence or absence of the fluorescent label, after dissociation of the hybridized nucleic acids, in the nucleic acid linked to the solid support, the labels presence indicating the **ligation** to the third nucleic acid to the second nucleic acid linked to the mobile solid support, the identity of the test **nucleotide** in the second nucleic acid indicating the identity of the selected **nucleotide**;

(5) a method of quantitating expression of a selected nucleic acid in a sample, comprising:

(a) contacting message nucleic acid isolated from a selected source with a nucleic acid probe linked at its 5' end to a detectably tagged mobile support, where the nucleic probe comprises a region complementary to a section of the selected nucleic acid;

(b) performing the detection method of one of the methods above; and

(c) quantitating the fluorescence indicating the quantity of the selected nucleic acid in the sample; and

(6) a method of detecting a result from an identification reaction to identify a selected **nucleotide** in a target nucleic acid, comprising:

(a) contacting a target **oligonucleotide** comprising a first complementarity region and a second complementarity region, where the second region is 5' of the first, and the first is complementary to a

section of the target nucleic acid directly 3' of and adjacent to the selected **nucleotide**, with a sample comprising the target nucleic acid;

(b) performing a selected identification reaction with the first hybridization product to determine the identity of the selected **nucleotide** where a selectively labeled detection product comprising the second region of the target **oligonucleotide** can be formed;

(c) isolating the detection product by contacting the detection product with a capture **oligonucleotide** covalently coupled to a mobile solid support; and

(d) detecting and/or identifying the label of the labeled detection product in the second hybridization product, the presence and/or identity of the label indicating the identity of the selected **nucleotide** in the target nucleic acid.

USE - The method is used to detect single nucleotide polymorphisms in genomic or amplified nucleic acid, e.g. for genotyping (detecting disease-associated mutations or pathogens), determining parentage, detecting or identifying pathogens and for determining differential gene expression.

ADVANTAGE - Exploiting the matrixing potential of mobile solid support (having multiple dye color/concentration capabilities) overcomes the limitations of known (immobilization-based) genetic bit analysis methods, i.e. low density on a two-dimensional support; photobleaching; auto-fluorescence of supports, inconsistent coupling of oligonucleotides to glass, and lack of flexibility, and expense, in producing new fixed arrays.

Dwg.0/0

L34 ANSWER 27 OF 34 WPIDS (C) 2002 THOMSON DERWENT
AN 1999-633727 [54] WPIDS
AB WO 9951621 A UPAB: 20020528

NOVELTY - Method for hybridizing two nucleic acids (NA) in which at least one NA comprises a minor groove binder (MGB)-**oligonucleotide** conjugate (A), is new. MGB is a molecule of 150-2000 D that binds in a non-intercalating manner to the minor groove of a double-stranded NA with association constant over 103 M-1.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(a) method of primer extension in which at least one primer is (A);

(b) differentiating between NAs that differ by a single **nucleotide** (nt) by reacting a target NA with at least two (A), one being perfectly complementary with the target and the other having a single mismatch, and determining hybridization strength of each (A);

(c) method similar to (b) but using single (A) of known sequence and at least two NAs, one the perfect complement of (A) and the other having a single nt mismatch;

(d) method for **ligating** two or more **oligonucleotides** (ON), each hybridized to adjacent positions on a target in which at least one ON is an (A);

(e) **oligonucleotide** probes (ONP) or use in mismatch discrimination and having one or more **detectable labels** that is an (A);

(f) (A) for use as a primer, having a 3'-end extendable by a polymerase;

(g) compositions and kits containing ONP of (e) or (A) of (f);

(h) primer-dependent nt sequencing using (A) as primer;

(i) method of examining gene expression using an array of ONP, at least one being an (A);

(j) method for identifying one or more mutations in a gene using an array of ONP as above;

(k) (A) that hybridize to a target NA to form a hybrid the melting

point (m. p.) of which is independent of base composition;

(l) method of cDNA synthesis using (A) as primer; and

(m) method for detecting a target NA by hybridization to (A) that is perfectly complementary to a target that differs by only a single nt from other non-target NAs.

ACTIVITY - None given.

MECHANISM OF ACTION - None given.

USE - Hybridization with (A), particularly where this is a probe or primer, is used in primer extension (amplification) reactions; to identify single-**nucleotide** (nt) mismatches; in ligase reactions; in sequencing; for analysis of gene expression and detection of mutations; for detecting target nucleic acids (especially for diagnosis or forensic analysis, e.g. to detect human immune deficiency virus or to differentiate between its subtypes, including those that are resistant to antiviral agents) and for cDNA synthesis.

ADVANTAGE - (A) form hybrids with complementary target sequences of very high stability, so even short probes, e.g. 8-mers, are highly specific and efficient. (A) also improve the discriminatory capacity of short **oligonucleotides**, providing better detection of single-base mismatches, and the speed (more rapid annealing to target) and versatility of assays are increased. Short primers are easier, and less expensive, to produce.

Dwg.0/5

L34 ANSWER 28 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2000:88512 Document No.: PREV200000088512. Expression profiling by iAFLP: A PCR-based method for genome-wide gene expression profiling. Kawamoto, Shoko; Ohnishi, Tadashi; Kita, Hiroko; Chisaka, Osamu; Okubo, Kousaku (1). (1) Institute for Molecular and Cellular Biology, Osaka University, 1-3, Yamada-oka, Suita, Osaka, 565 Japan. Genome Research, (Dec., 1999) Vol. 9, No. 12, pp. 1305-1312. ISSN: 1088-9051. Language: English. Summary Language: English.

AB The availability of comprehensive sets of genes has prompted the researchers to carry out systematic collection of gene expression data. RT-PCR has the highest specificity and sensitivity for transcript detection among all available methods. Low throughput, especially when quantitative data are desired, has precluded RT-PCR from genome-wide application. Here we report a PCR-based expression profiling method, introduced amplified fragment length **polymorphism** (iAFLP), that has the same specificity and sensitivity as RT-PCR and a throughput level comparable to that of **DNA**-microarray hybridization. In this method, restricted ends of total cDNAs from six sources were **ligated** with adaptors having various length of short insertions to a common sequence (polymorphic adaptors). Amplification of a pool of these differentially adapted cDNAs with a gene-specific primer and an adaptor primer allows us to quantitate the abundance of any transcript in six mRNA sources. Using three different primer colors this technique allows quantitation of expression of 864 genes across six different sources per day with a single autosequencer, which is comparable to the throughput of microarray analysis in terms of number of genes X number of sources.

L34 ANSWER 29 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2000:95825 Document No.: PREV200000095825. **DNA** technology in the clinical laboratory: Overview. Kiechle, Frederick L. (1). (1) Department of Clinical Pathology, William Beaumont Hospital, 3601 W 13 Mile Rd, Royal Oak, MI, 48073-6769 USA. Archives of Pathology & Laboratory Medicine, (Dec., 1999) Vol. 123, No. 12, pp. 1151-1153. ISSN: 0363-0153. Language: English. Summary Language: English.

AB Objectives.-To review the advances in clinically useful molecular biological techniques and to identify their applications in clinical practice, as presented at the Eighth Annual William Beaumont Hospital Symposium. Data Sources.-The 10 manuscripts submitted were reviewed, and

their major findings were compared with literature on the same topic. Study Selection.-Two manuscripts addressed specimen (nucleic acid) stability, 2 described novel analytic approaches, 3 discussed detection of B- or T-cell clonality in lymphoproliferative disorders, and 3 reported the frequency of a variety of genetic **polymorphisms** found in cardiac disorders. Data Synthesis.-**DNA** from dried blood spots is stable and may be purified rapidly for amplification and mutation analysis. **RNA** is much less stable, and a variety of methods may be used to reduce ribonuclease degradation of enteroviral **RNA**. False-negative reactions may be reduced by genomic amplification of **ligated** padlock probes by cascade rolling circle or polymerase chain reaction. A multiplex polymerase chain method using fluorescence-labeled products that separate both the wild-type and mutant hemochromatosis gene alleles by capillary gel electrophoresis represents another approach for detecting the 2 major missense mutations (C282Y and H63D) in hemochromatosis. Southern blotting and polymerase chain reaction have been used to detect B- and T-cell clonality in lymphoproliferative diseases, including mantle cell lymphoma and lymphoma of the breast. Genetic **polymorphisms** in a variety of coagulation factors and platelet glycoprotein IIIa are associated with ischemic heart disease. Conclusions.-As the Human Genome Project continues to define disease-associated mutations, the number of clinically useful molecular pathologic techniques and assays will expand. Clinical outcome analysis is still required to document a decrease in the patient's length of stay to offset the cost of introducing molecular biological assays in the routine clinical pathology laboratory.

L34 ANSWER 30 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:227326 Document No.: PREV199900227326. Modification of the AFLP protocol applied to honey bee (Apis mellifera L.) **DNA**. Suazo, Alonso; Hall, H. Glenn (1). (1) Department of Entomology and Nematology, University of Florida, Gainesville, FL, 32611 USA. Biotechniques, (April, 1999) Vol. 26, No. 4, pp. 704-709. ISSN: 0736-6205. Language: English. Summary Language: English.

AB The established amplified fragment-length **polymorphism** (AFLP) protocol was simplified and optimized for honey bee **DNA** (Apis mellifera L.). Compared to the original method, the following simplifications were made: (i) the digestion of **DNA** and **ligation** of the adapters are performed in one reaction vs. two, (ii) one restriction enzyme is used vs. two and (iii) amplification is accomplished in one reaction vs. two. PCR products are resolved in agarose-Synergel instead of polyacrylamide and are visualized by ethidium bromide staining rather than by autoradiography of labeled primers. Using the modified procedure for honey bee **DNA**, high reproducibility of the band patterns of PCR products and low sensitivity to the amplification conditions were seen. Analysis of honey bee **DNA** revealed considerable genetic variability within and between African and European bee samples. African- and European-specific fragments were found.

L34 ANSWER 31 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:271311 Document No.: PREV199800271311. A homogeneous, ligase-mediated **DNA** diagnostic test. Chen, Xiangning; Livak, Kenneth J.; Kwok, Pui-Yan (1). (1) Div. Dematol., Washington Univ. Sch. Med., Saint Louis, MO 63110 USA. Genome Research, (May, 1998) Vol. 8, No. 5, pp. 549-556. ISSN: 1088-9051. Language: English.

AB Single-**nucleotide** variations are the most widely distributed genetic markers in the human genome. A subset of these variations, the substitution mutations, are responsible for most genetic disorders. As single **nucleotide polymorphism** (SNP) markers are being developed for molecular diagnosis of genetic disorders and large-scale population studies for genetic analysis of complex traits, a simple, sensitive, and specific test for single **nucleotide** changes is

highly desirable. In this report we describe the development of a homogeneous **DNA** detection method that requires no further manipulations after the initial reaction is set up. This assay, named dye-labeled **oligonucleotide ligation** (DOL), combines the PCR and the **oligonucleotide ligation** reaction in a two-stage thermal cycling sequence with fluorescence resonance energy transfer (FRET) detection monitored in real time. Because FRET occurs only when the donor and acceptor dyes are in close proximity, one can infer the genotype or mutational status of a **DNA** sample by monitoring the specific **ligation** of dye-labeled **oligonucleotide** probes. We have successfully applied the DOL assay to genotype 10 SNPs or mutations. By designing the PCR primers and **ligation** probes in a consistent manner, multiple assays can be done under the same thermal cycling conditions. The standardized design and execution of the DOL assay means that it can be automated for high-throughput genotyping in large-scale population studies.

L34 ANSWER 32 OF 34 HCA COPYRIGHT 2002 ACS
127:215947 Detection of nucleic acid sequence differences using the ligase detection reaction with addressable array. Barany, Francis; Barany, George; Hammer, Robert P.; Kempe, Maria; Blok, Herman; Zirvi, Monib (Cornell Research Foundation, Inc., USA; University of Minnesota; Louisiana State University; Barany, Francis; Barany, George; Hammer, Robert P.; Kempe, Maria; Blok, Herman; Zirvi, Monib). PCT Int. Appl. WO 9731256 A2 19970828, 124 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US1535 19970205. PRIORITY: US 1996-11359 19960209.
AB The present invention describes a method for identifying one or more of a plurality of sequences differing by one or more single base changes, insertions, deletions, or translocations in a plurality of target **nucleotide** sequences. The method includes a **ligation** phase, a capture phase, and a detection phase. The **ligation** phase utilizes a **ligation** detection reaction between one **oligonucleotide** probe, which has a target sequence-specific portion and an addressable array-specific portion, and a second **oligonucleotide** probe, having a target sequence-specific portion and a **detectable label**. After the **ligation** phase, the capture phase is carried out by hybridizing the **ligated oligonucleotide** probes to a solid support with an array of immobilized capture **oligonucleotides** at least some of which are complementary to the addressable array-specific portion. Following completion of the capture phase, a detection phase is carried out to **detect** the **labels** of **ligated oligonucleotide** probes hybridized to the solid support. The **ligation** phase can be preceded by an amplification process. The present invention also relates to a kit for practicing this method, a method of forming arrays on solid supports, and the supports themselves.

L34 ANSWER 33 OF 34 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:302276 Document No.: PREV199800302276. Physical and linkage mapping of the bovine bone morphogenetic protein 1 on the evolutionary break region of BTA8. Martin-Burriel, I. (1); Goldammer, T.; Elduque, C.; Lundin, M.; Barendse, W.; Zaragoza, P.; Olsaker, I.. (1) Lab. Genet. Bioquim., Fac. Vet., Miguel Servet 177, 50013 Zaragoza Spain. Cytogenetics and Cell Genetics, (1997) Vol. 79, No. 3-4, pp. 179-183. ISSN: 0301-0171. Language: English.
AB Bone morphogenetic protein 1 (BMP1) has been proposed as a regulatory